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A PRACTICAL COURSE IN AGRICULTURAL CHEMISTRY

A PRACTICAL COURSE
IN
AGRICULTURAL CHEMISTRY

FOR SENIOR STUDENTS OF AGRICULTURE, DAIRYING, HORTICULTURE AND POULTRY HUSBANDRY

BY

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AND

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LATE LECTURER IN AGRICULTURAL CHEMISTRY, ESSEX INSTITUTE OF AGRICULTURE, WRITTLE

WITH A FOREWORD BY

SIR JOHN RUSSELL, D.Sc., F.R.S.

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FOREWORD

By Sir John Russell, D.Sc., F.R.S.
Director of the Rothamsted Experimental Station.

The authors of this book need no introduction to agricultural experts and agricultural students. Their association with the Chemical Department of the Essex Institute of Agriculture, one of the oldest and best known Agricultural Institutes in this country, has brought them in contact with large numbers of agriculturists, and has given them a wide knowledge of the educational needs of agricultural students and the best ways in which these can be met.

The authors have the further advantage of being themselves research workers in soils and plant growth, so that they are in a position to view the problems as a whole, right from the research laboratory to the farm. In this book the treatment is throughout sufficiently elementary for the young student, yet it has been so designed that he will have nothing to unlearn if he elects to take up a scientific or advisory career. A further valuable feature is that explanations are given not merely of the processes performed, but of the relation of the experiment to the whole subject, so that the student may be able to appreciate the bearing of his laboratory exercises on farm practice.

The book will be of great help to agricultural students, and will add to the already high reputation of the authors and of the Institute with which they are connected.

E. J. RUSSELL.

Rothamsted Experimental Station,
Herts, *May* 1936.

PREFACE

THIS book has been produced to meet the requirements of students preparing for the B.Sc. degrees in Agriculture, Horticulture and Dairying, for the National and College diplomas in these subjects, and in Poultry Husbandry.

Much of the work in practical chemistry is common to all these examinations, but there are necessarily differences in the emphasis placed on the various branches. The student taking a degree or diploma in Agriculture is expected to have a general knowledge of the whole ground covered by the book and to pay greater attention to some sections more vitally affecting Agriculture, *e.g.* feeding stuffs. The Poultry student, however, will probably omit the sections on soils, dairying and insecticides. The Dairy student likewise will omit the sections on soils and insecticides.

The experience of the writers with their own students has convinced them that the latter require a book which contains, in suitable form, a considerable amount of material at present only accessible in scientific journals, specialised books and miscellaneous publications, and which has discarded obsolete methods and views.

In the absence of a suitable book, it has been our practice for many years to issue typed sheets of instructions for laboratory work which have periodically received revision and addition. These sheets form the nucleus of the present book, in which the larger amount of annotation possible should have the twofold effect of making laboratory work more nearly appear in its rightful perspective, and of reducing the time occupied by mechanical note-taking in lecture periods.

The student is assumed to possess a fair knowledge of the common qualitative and quantitative processes of general chemistry, since this is normally acquired in the years pre-

ceding the final training in the branch of applied chemistry demanded by the examination syllabus.

The book contains a considerable number of quantitative exercises, and makes no attempt to cater for the needs of students whose complete course in all subjects extends over a few weeks or months only, although many of the qualitative exercises could be worked through by such students with advantage.

As mentioned earlier, the scientific literature and existing text-books have been freely consulted in the preparation of the book.

The authors' thanks are due to Mr. F. W. Hendry for preparing the illustrations and for help in correcting the proofs, and to Mr. H. W. Gardner for his helpful criticism of the manuscript.

F. K.
J. E. W.

CHELMSFORD, *April* 1936.

PREFACE TO THE SECOND EDITION

THE opportunity has been taken to revise the text and to include new matter which the advancement of agricultural science has made desirable. Several qualitative tests have been superseded by newer ones which are more reliable or easier to apply. The quantitative exercises have been similarly treated, notably with regard to milk proteins and soil organic matter.

Where possible, the quantitative exercises follow the methods of analysis officially prescribed, but the book is in no sense intended as a reference book for the practising chemist although it is hoped it may not be without interest to him.

We have felt compelled to keep before us the limited time which can usually be given to the subject by the student for whom the book is intended, and accordingly we have not hesitated to choose methods, or simplify others, to meet his need, provided that reasonable accuracy is obtainable and no loss of fundamental principle is involved.

Under certain circumstances, and under guidance of his teacher, it may not be considered necessary for the student to carry out all the exercises given in a section of the book. The exercises are sufficiently numerous to allow of choice, but it will be evident that the book could be expanded to an extent which would place it beyond the purse and purpose of those for whom it is intended.

F. K.
J. E. W.

WRITTLE, *February* 1946.

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CHAPTER I

SOILS

In the early days of agricultural chemistry, it appeared reasonable to hope that the simple chemical analysis of soils would unmistakably reveal specific causes of infertility and lead to the growth of larger crops. Although this aim is at present without complete realisation, the intervening years have been fruitful by bringing a broader outlook and a much fuller appreciation of the complexity of soil problems, and in particular of those problems which involve the relationship between soil and plant. Much of a practical nature has, however, been accomplished, for the causes of infertility in large numbers of soils are clearly revealed by laboratory tests. Further progress will follow the correlation of laboratory work with the results of reliable field trials.

The academic worker may be profoundly interested in soil as such, and regardless of its crop potentialities, and the farmer may be solely interested in his soil from the point of view of its crop-producing power, but between the two is the farmer's scientific adviser, whose soil studies are made with an immediate utilitarian object. There is, moreover, an educational aspect of such soil studies, for in few subjects is it so difficult to obtain the essential view from behind the scenes, to enable the tangled skein of factors which make up soil fertility to be unravelled.

The exercises described in this section illustrate the methods by which the agricultural properties of soils are studied in the laboratory, with sufficient annotation to enable the student to appreciate the educational value of the work.

It is, however, important that knowledge gained in the laboratory should be supplemented by observations on soil structure, variation in texture, reaction, etc. made in the field under the guidance of an experienced person—a form of

instruction which written notes can hardly replace and which is impossible to give within the confines of size and scope of this book. Consequently the value of the laboratory work is greatly enhanced if conducted upon soil samples taken during the course of field study.

SAMPLING AND PREPARATION OF SAMPLE

Surface samples of soil are usually taken to a depth of nine inches, unless there is a marked difference between soil and subsoil before that depth is reached. The subsoil is usually taken as the depth 9 to 18 inches or the next nine inches following the change from soil to subsoil. In special cases, *e.g.* fruit soils, deeper samples may be taken, and in the study of soil profiles, important from the point of view of soil survey work, samples are taken from each level or " horizon " from the surface to the parent rock. It is important that several samples should be taken from the same field or uniform area, and that these be bulked and well mixed before being analysed. In practice, little is to be gained from analysing each sample separately and averaging the results, for the experimental errors are very little larger when only one analysis of the composite sample is carried out.

Various sampling tools are used, the most convenient being a 2-inch auger or a special cylindrical tube made for the purpose. The latter consists of a steel tube 2 inches in diameter and 12 inches long. It has a $\frac{3}{4}$-inch slit cut lengthwise and all its edges are sharpened. The tube is fixed to a vertical steel rod bent at the end to a ring 2 inches in diameter, through which a wooden handle is fixed. The core of soil obtained is removed with a pointed iron rod. In many cases a spade or trowel may also be used to take the samples.

In the laboratory, the samples of soil are spread out in shallow trays to dry. When air-dry, the soil is sieved through a 2 mm. sieve to remove larger particles of vegetable matter and stones. The residue from the sieve is rubbed up in a mortar with a wooden pestle, care being taken not to crush stones, and the material is again sieved. The soil passing through the sieve is called " fine earth " ; it is well mixed and is used for subsequent analysis. The treatment which it undergoes afterwards will depend upon the purpose for which it is required ; *e.g.* for some of the chemical analyses a sub-sample of the soil is ground as finely as possible in order to obtain a small representative sample for the ultimate analysis.

PLANT NUTRIENTS IN THE SOIL

Most soils contain adequate amounts of the plant nutrients, but not necessarily in a form in which they are available for plants. The presence of these constituents in a soil may be tested for qualitatively.

Nitrogen. (*a*) *Organic.* Heat about 2 gm. of soil with an equal weight of soda lime in a test tube. Note that ammonia is given off.

(*b*) *Nitrates.* To about 20 gm. of soil add 100 ml. of distilled water and shake for 5 minutes. Allow the soil to settle, filter off the clear liquid and evaporate to a small bulk. To the residue when cool add a little pure strong sulphuric acid and pour into a test tube containing a little diphenylamine reagent. The formation of a blue colour indicates the presence of nitrates.

Phosphate. Ignite a few gm. of soil in a basin, cool, and then boil with 10 ml. of strong nitric acid. Cool, add an equal volume of water and filter. To the filtrate add ammonium molybdate solution and warm. A yellow precipitate indicates the presence of phosphate.

Potassium. Boil about 10 gm. of soil with 25 ml. of dilute hydrochloric acid for 5 minutes. Filter, evaporate the filtrate to dryness and ignite the residue. Cool, and extract the residue with hot water and again filter. To the filtrate add sodium cobaltinitrite solution. A yellow precipitate indicates the presence of potassium.

Iron. Boil about 10 gm. of soil for 5 minutes with 20 ml. of strong hydrochloric acid. Dilute with an equal volume of water and filter. Test a portion of the filtrate for iron by the addition of potassium ferrocyanide or potassium thiocyanate solution. A dark blue or blood red coloration respectively indicates the presence of iron.

Calcium. Take the remainder of the filtrate from the iron test, boil with a little strong nitric acid, and add ammonium chloride and excess of strong ammonia to precipitate iron, etc. Filter, concentrate the filtrate and add ammonium oxalate solution. A white precipitate indicates the presence of calcium.

ABSORPTION OF SUBSTANCES BY SOILS

Certain substances are absorbed and retained by soils, and they can be conveniently divided into three classes : (1) cations

(bases), (2) anions (acid radicles), and (3) colloidal matter, especially organic matter.

Absorption of Cations. This is now regarded simply as an exchange of cations, *e.g.* if K or NH_4 ions are absorbed by the colloidal soil particles, equivalent amounts of Ca or Mg or Na ions are replaced.

Absorption of Anions. This is most probably due to chemical precipitation, *e.g.* phosphates form insoluble salts. The anions that are not absorbed, HCO_3, SO_4, NO_3 and Cl, do not form insoluble salts with the soil bases at their concentrations in the soil.

Absorption of Organic Matter. This is mainly due to precipitation of the organic matter in a manner similar to that which occurs in the flocculation of clay. It is of great importance in soil fertility for practically all of the organic matter added to soil by plant residues and as dung remains near the surface. Again this property is made use of in the purification of sewage on sewage farms.

Absorption of Salts (Qualitative). Cover the bottom of a lampglass with a piece of muslin, and fill with a clay soil to a depth of about 8 inches.

Prepare a solution containing 0·1 per cent. each of ammonium sulphate, potassium chloride, sodium phosphate and sodium nitrate. Pour the solution on to the soil, allow to drain through and collect the filtrate. Carry out comparative tests on the original solution and on the solution after passing through the soil, care being taken to use the same volumes of solution and of reagents in each case.

(*a*) *Ammonia.* Mix 1 ml. of each solution with a little oxalic acid solution, make up to 100 ml. and allow the precipitated calcium oxalate to settle. By means of a pipette transfer 50 ml. of the clear liquid to a Nessler cylinder, mix with 2 ml. of Nessler's reagent, and allow to stand for 5 minutes. Note the depth of colour produced in each case.

(*b*) *Nitrates.* Take 5 ml. of each solution and evaporate nearly to dryness. Cool, mix with pure strong sulphuric acid and a drop of diphenylamine reagent. Note the depth of the blue coloration in each case.

(*c*) *Potassium.* Acidify equal quantities of the two solutions with acetic acid, and test with a fresh solution of sodium cobaltinitrite. Note the extent of the yellow precipitate in each case.

(*d*) *Phosphate.* Acidify equal quantities of both solutions with a few drops of nitric acid. Add ammonium molybdate solution, warm, and compare the amount of precipitate produced in each case.

FLOCCULATION AND DEFLOCCULATION OF CLAY

The finest particles of a soil—that is those of diameter 0·002 mm. or less—make up the clay fraction of a soil. Most of these particles have colloidal properties, and under certain conditions may be aggregated or coagulated to form larger ones, which themselves act as separate soil particles. This phenomenon, which is termed flocculation, is most important from the point of view of the cultivation of clay soils, where it is necessary to get the soil in a coarse-grained condition in order to obtain the required tilth. The reverse condition, *i.e.* deflocculation of the clay particles, occurs when the soil remains persistently wet, and " puddles " when worked. Flocculation of the clay particles is produced by electrolytes such as salts and acids, and in the field lime or chalk is added for the purpose of flocculating the clay. Deflocculation is effected by alkalies and occurs in " alkali " soils, while in the field heavy dressings of certain manures such as nitrate of soda, which leave an alkaline residue in the soil, may deflocculate the clay.

Effect of Acids, Alkalies and Salts. Rub up a few gm. of clay soil with distilled water in a mortar and dilute the thin paste to about a litre. Allow to stand for a few hours and pour off the turbid suspension into another vessel. Shake well and pour off into a number of 100 ml. cylinders, which should be treated as follows :

(*a*) Nothing added—used as control.
(*b*) 1 ml. of 0·5N. HCl.
(*c*) 1 ml. of 0·5N. NaCl solution.
(*d*) 1 ml. of 0·5N. $CaCl_2$ solution.
(*e*) 5 ml. of lime water.
(*f*) 5 ml. of a saturated $Ca(HCO_3)_2$ solution.
(*g*) 1 ml. of 0·5N. NaOH solution.

Shake the cylinders again and note the respective times taken for the clay to flocculate, as shown by the liquid becoming clear, with the aggregated particles settled out to the bottom of the cylinder.

DENSITY AND PORE SPACE

Soils have a " true " density, which is that of the soil particles, and also an " apparent " density, which is that of the soil system, including the pore space. The apparent density and the pore space are closely related, both being dependent on the degree of packing, while the apparent density will also depend on the degree of saturation of the pore space with water.

True Density of Soil. Weigh a 100 ml. specific gravity bottle, and weigh it again when filled with distilled water. Weigh 10 gm. of air-dry soil into a small beaker and boil for a short time with a few ml. of water in order to expel air. Empty the bottle of water and wash the soil into the bottle with a jet of distilled water. Allow to cool to the temperature of the air, fill up with water and weigh. The weight of soil used divided by the weight of water displaced gives the specific gravity of the soil (for practical purposes this is equivalent to the density).

In this experiment the assumption is made that the soil displaced its own volume of water. This is not quite correct because the soil colloids absorb water and in doing so increase in volume.

Apparent Density. Weigh a large weighing bottle without the stopper. Fill with soil flush with the brim and weigh again. Find the volume of the bottle by measuring the volume of water needed to fill it. The apparent density of the soil is obtained by dividing the weight of the soil by the volume.

Pore Space by Calculation. It is difficult to determine the pore space by direct measurement, but it may be calculated from the true and apparent density of the soil.

Let d = true density and d_1 = apparent density.

Suppose volume of soil = v.

Then weight of soil $= v \times d_1$.

But the volume of soil particles $= \frac{v \times d_1}{d}$.

$\therefore$ Volume of pore space $= v - \frac{vd_1}{d} = \frac{v(d - d_1)}{d}$.

$\therefore$ Percentage of pore space $= \frac{v(d - d_1)}{d} \times \frac{100}{v} = \frac{d - d_1}{d} \times 100$.

STICKY POINT

The character of a soil is the resultant of many interacting factors, *e.g.* proportions of different sizes of particles, amounts of clay and other colloids, calcium carbonate and soluble salts, etc. It is, therefore, impossible to describe a soil adequately by a small number of figures which give the results of certain not necessarily connected measurements. However, some " single value " determinations, such as the sticky point, are helpful in assessing soil texture.

The sticky point depends upon the proportion of colloidal matter in the soil, and may be regarded as the moisture content of the soil at which the colloidal components of the soil are saturated with water.

The values for sticky point and " moisture equivalent " (another single value physical constant) are closely related in mineral soils containing more than about 12 per cent. of clay, the figure for sticky point being in general slightly higher than that for " moisture equivalent ". The determination of the latter is not described, as little would be gained if sticky point is determined.

Spread out about 10 gm. of soil in a thin layer on a glass plate, and spray with a fine jet of distilled water from a wash bottle until the soil is thoroughly wet and sticky. Work up the mass into a paste with a flexible steel spatula. Scrape the soil from the glass plate and knead it in the fingers until it ceases to adhere to them or the spatula. At this stage it is possible to cut clean through the plastic mass without the soil adhering to the spatula. Place in a weighed dish and weigh as quickly as possible. Dry to constant weight in a steam oven, and calculate the moisture content on a dry soil basis.

At the point of stickiness the moisture is not all associated with the soil colloids. A part of it, amounting to about 20 per cent. of the weight of the sand content, occurs as films and wedges between the soil particles. To arrive at a better figure

for assessing soil texture, a correction should be made, allowing for the sand content. The corrected figure is :

$$\text{Moisture content at sticky point} - \frac{\text{Percentage of Sand}}{5}.$$

This value is termed the "index of texture"; for sand the value is 0, while the upper limit for clay soils is about 55.

WATER HOLDING CAPACITY

The water holding capacity is the amount of water taken up by dry soil, when immersed in water under standardised conditions. It is of value for pot culture work, since it provides a simple means of determining moisture contents required in pot soils for good plant growth. Thus with medium textured soils in pots, good growth occurs at moisture contents corresponding to 50-70 per cent. of the total water holding capacity of the soil.

The determination is carried out in a small circular brass box with a diameter of approximately 5 cm. and height of 1·6 cm. and with a perforated base.

Crush the air-dry soil in a mortar, avoiding grinding sand grains, and continue grinding until no more will pass through a sieve having round holes 0·5 mm. in diameter. Add the coarse particles on the sieve to the fine particles and mix thoroughly. Place a filter paper in the bottom of the small circular box and weigh. Add soil in small quantities at a time and tap the box frequently so that the soil packs evenly. Continue adding soil, tapping until the box is nearly full, then add enough soil to fill the box and strike off the surplus until level with the top of the box. Tap the box smartly and if necessary again add soil and level off. Place the box and contents in a Petri dish, add water to the dish until the depth of water is one-quarter of an inch and leave overnight. During the absorption of water, considerable movement of the soil may take place, but if some particles are pushed over the side this does not matter. Remove the box, wipe the outside dry and weigh immediately. After weighing, place in an oven at 105° C. and dry for 24 hours, or until the weight is constant. Cool in a desiccator and weigh. Correct for the moisture taken up by the filter paper, by weighing six papers,

saturating them with water and removing the surplus with a glass rod. Weigh again and calculate the average amount of water retained. Calculate the water holding capacity as a percentage of the oven-dry soil.

FIELD CAPACITY

Field capacity is the amount of water held in a soil after the excess of gravitational water has drained away and the downward movement of water has practically ceased. The value for field capacity is less than for water holding capacity, since the latter measures the moisture present in a fully saturated soil resting on a water table, so that the soil pores are completely filled with water. For very light soils the field capacity is generally higher than the moisture present at the sticky point, while for medium to heavy soils the values are similar.

After rainfall sufficiently heavy to saturate the soil, cover the soil surface to prevent evaporation or further wetting. After two to three days take several samples with an auger, transfer quickly to a tin with a closely fitting lid and transport to the laboratory as quickly as possible. Weigh about 50 gm. and dry in an oven at 105° C. until constant in weight. Calculate the field capacity, which is the weight of moisture lost on drying expressed as a percentage of the oven-dry soil remaining.

HUMUS

The term " humus " is used in more than one sense, ranging from the whole of the organic matter in the soil to certain fractions of it such as that which is soluble in dilute alkali solutions, or to the complex formed on precipitation of the alkaline extract by acids. In the practical sense we may regard it as including all the organic matter sufficiently decomposed to form colloidal complexes in the soil. Its importance in the soil in contributing to a good soil structure, water holding capacity and base exchange, is well established. The following experiment demonstrates the presence of a " humus " fraction soluble in alkali and precipitated by acids—often called " humic acids ".

Treat 50 gm. portions of a mineral soil and a peaty soil with about 100 ml. of dilute hydrochloric acid (5 ml. acid to 100 ml. water). Bring to the boil, filter and wash the soil with hot water until free from acid. (This preliminary treatment ensures the breaking down of insoluble "humates", *e.g.* "calcium humate", into free " humic acids ".) Transfer the extracted soil to a beaker, add about 100 ml. of dilute ammonia (about 5 per cent.), stir well and boil for a few minutes. Filter and add sufficient warm dilute hydrochloric acid to the filtrate to give a slight excess of acid. Filter off the flocculent precipitate (humus) and dry. Compare the amount of " humus " obtained from the two soils.

AMMONIFICATION AND NITRIFICATION

Complex nitrogenous substances including part of the soil humus and organic nitrogenous manures, undergo several changes in the soil before the nitrogen becomes available for plants. The change to ammonia is brought about by various species of bacteria and fungi. The ammonia formed by soil bacteria or added in manures is converted by other bacteria first to nitrite and lastly to nitrate. The latter change is extremely rapid, with the result that very little nitrite is ever found in normal soils. The following experiments demonstrate qualitatively the power of soil to produce ammonia from organic nitrogen and to produce nitrate from ammonium compounds.

Ammonification. Make an intimate mixture of equal parts of soil and sand, divide into two portions of approximately 200 gm. each and to one portion add 2 gm. of dried blood and mix well. Place the separate portions in beakers and to each beaker add about 50 ml. of distilled water or sufficient to just moisten all of the sand-soil mixture. Keep in an incubator at about 37° C. for a week, adding more water at intervals to keep the soil moist. Then test the contents of each beaker for ammonia as follows. Remove an equal amount of the contents, transfer to a flask, add about 100 ml. of 0·3N. hydrochloric acid, shake for one minute and filter. To 50 ml. of the filtrate or a suitable aliquot diluted to 50 ml. add 2 ml. of Nessler solution and compare the amount of ammonia present in each case.

Nitrification. Prepare a nutrient solution containing 0·5 gm. ammonium sulphate, 0·5 gm. sodium chloride, 0·25 gm. potassium phosphate, 0·2 gm. magnesium sulphate and 0·1 gm. ferrous sulphate in 1 litre of water. Place 100 ml. of this solution into each of two small flasks and add 0·5 gm. of calcium carbonate to each. Boil for a few minutes, allow to cool and add about 0·2 gm. of an arable soil to one flask. Plug with cotton wool and place in an incubator at 37° C. or in a warm place in the dark. Test a few ml. of the solution from each flask for nitrate with diphenylamine solution at intervals of a week for four weeks. At the end of the period test the solution in each flask for ammonia, and note the great reduction in amount in the flask to which the soil had been added.

SOIL SOURNESS, SOIL ACIDITY AND METHODS FOR DETERMINING THE LIME STATUS OF SOILS

Sourness is a condition of infertility in soils which can be corrected by the application of lime or chalk. Sour soils are always acid, or in modern terminology have a high degree of unsaturation. All unsaturated soils are not necessarily acid, for this will depend on the proportion of metallic cations (exchangeable bases), especially of calcium, to hydrogen ions which are present in the colloidal soil particles.

Hydrogen ion Concentration or pH value. The intensity of acidity of a solution is dependent upon the concentration of hydrogen ions (H^+) present. This, like any other number, can be stated as 10 raised to some power, *e.g.* 10^{-7}, 10^{-6}, 10^{-8}, etc. It has, however, become customary to use the logarithm of the number and omit the negative sign, and to designate this notation by the symbol *p*H.

Thus $$p\mathrm{H} = -\log(\mathrm{H}^+) = \log\frac{1}{(\mathrm{H}^+)}$$

and when $(\mathrm{H}^+) = 10^{-7}$, $p\mathrm{H} = 7$,

$(\mathrm{H}^+) = 10^{-6}$, $p\mathrm{H} = 6$,

$(\mathrm{H}^+) = 10^{-8}$, $p\mathrm{H} = 8$.

Using this notation, we have a complete scale of acidity or alkalinity in which *p*H 7 represents neutrality, solutions with

*p*H values less than 7 are acid, and those with *p*H values of more than 7 are alkaline. A difference of *p*H of one represents a difference of ten times in hydrogen ion concentration.

Electrometric Method. Electric currents pass through solutions by means of the ions present. The greater the degree of ionisation in a solution, the more readily will the current pass and the less will be the resistance. It follows that a measure of the resistance can be used to measure the concentration of hydrogen ions. For this purpose the resistance is balanced against a known variable resistance, by an instrument known as a potentiometer.

Fundamentally the electrode should be a " hydrogen electrode " which in actual practice consists of a piece of platinum covered with a layer of minute bubbles of hydrogen. When two such electrodes separately dip into solutions of different *p*H values and the two containing vessels are connected by a " fluid bridge " a " concentration cell " is formed by the two " half cells " and a difference of electrical potential is established between the electrodes. This potential difference can be measured by noting the extent to which the potentiometer has to be adjusted in order that no current shall pass.

The hydrogen electrode, however, is inconvenient to use and is subject to the influence of various substances which " poison " it and in consequence other electrodes have replaced it. For soils, the glass electrode is generally used, as it is little subject to errors due to disturbing substances present in some soils and it is also simple to operate.

The fundamental principle of this electrode is that a very thin glass membrane allows certain ions to pass through it and if such a membrane is used to separate solutions of definite hydrogen ion concentrations, an EMF is set up upon it. If the *p*H of one of the solutions is known the *p*H of the other solution can be calculated from a measurement of the EMF ; usually, however, no calculation is required since the instrument can be calibrated as a *p*H meter, *i.e.* to give direct readings of *p*H. The glass electrode is used in conjunction with a calomel

electrode (consisting of mercury and calomel covered with potassium chloride solution of definite concentration) the potential of which has been determined by direct reference to the hydrogen electrode. The potential of the glass electrode must be determined at least once on each day it is used, a process employing buffer solutions of known *p*H. With the *p*H meter this process is referred to as standardisation of the instrument.

The hydrogen ion concentration of a soil varies with the soil-water ratio used, and in this country the soil-water ratio of 1 to 2·5 recommended by the International Society of Soil Science is generally adopted.

Determination of pH. Under supervision standardise a *p*H meter with a buffer solution of known *p*H. Weigh 6 gm. of soil into the electrode vessel of the meter, add 15 ml. of distilled water and stir vigorously with a glass rod for one minute. Alternatively, place soil and water in the above proportion in a wide-mouth bottle, cork, shake vigorously for one minute and then transfer the suspension to the electrode vessel. Read off the *p*H of the soil.

Colorimetric Method. In the colorimetric method for determining the *p*H values of soils, the colours produced by soil extracts with suitable indicators are compared with the colours produced by adding the same indicators to buffer solutions of known *p*H values, or with permanent colour standards of tinted glass. For the determination, the water extracts should be as colourless as possible. Filtration cannot be used, as this affects the reaction of the solution, but suitable soil extracts may be prepared either by centrifuging or by Kühn's method by clearing the soil suspensions with specially prepared barium sulphate. The method to be described assumes that a " comparator " with glass standards, or buffer tubes, are available, and that barium sulphate is used for clearing the soil suspensions.

Carry out the test in test tubes of a size suitable for use with glass standards or buffer tubes. Test tubes of ½ inch diameter are suitable, and these should be marked as in the diagram ; the divisions *A*, *B*, *C* and *D* representing equal volumes, and the volume of *D* to *E* being approximately three times the volume up to *D*. For a sandy

soil fill two tubes to mark *A* with barium sulphate, to mark *D* with soil, and to mark *E* with distilled water (which has been recently boiled to expel carbon dioxide). To one of the tubes add about 0·5 ml. of an appropriate indicator, viz. bromo-cresol green or bromo-thymol blue, according to the acidity of the soil, or chloro-phenol red may be used over a wider range of acidity. The amount of indicator to add will depend on the soil, and should be varied so as to give a depth of colour similar to that of the colour standard. Shake the tubes vigorously until the contents are thoroughly mixed; allow to stand a few minutes, when the suspensions should have settled, leaving a clear upper layer. Compare the tube containing the indicator with the colours of the comparator or standard buffer tubes, placing the tube with no indicator behind the colours of the standards or buffer tube with indicator. In this way the colours are matched under similar conditions, the *p*H value being directly indicated by the number of the standard of the same colour, or by the *p*H of the standard buffer tube.

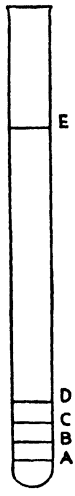


FIG. 1.—Test tube marked for *p*H measurement of soil.

For loams and clay soils more barium sulphate in proportion to soil may be necessary, the optimum proportions being found by trial and error. If the suspension settles rapidly, but the supernatant liquid is not clear, more barium sulphate and less soil must be used, while if the suspension clears slowly, too little water may be present. For reliable results, as much soil as possible, consistent with obtaining a clear solution, should always be used.

N.B.—Specially prepared barium sulphate for soil testing is used in the test. Some samples may be alkaline and each new supply should be carefully tested with soils of known *p*H to see that it gives reliable results.

Valuable information may be obtained in the field by the use of a mixed indicator directly added to a little soil. In this way variations in *p*H in different parts of the field may be detected and the cause of many crop failures ascertained.

Comber Test. When a neutral salt solution is added to an acid soil, an exchange of the cations of the salt occurs with the

calcium, hydrogen, and other cations of the soil, the result being that the solution becomes acid. Aluminium and iron from the soil are thereby brought into solution, and if potassium thiocyanate is used as the neutral salt, the CNS ion will combine with the iron to form ferric thiocyanate, which gives a red colour to the solution. This is made use of in the test devised by Comber.

Take 5 to 10 gm. of air-dry fine earth in a dry test tube and add 10 to 15 ml. of a saturated alcoholic solution of potassium thiocyanate. Shake well so that the soil becomes thoroughly mixed with the solution. The soil soon settles out, leaving a clear solution, which in sweet soils is colourless, but in acid soils is coloured red, the intensity of which is dependent on the soil. The depth of colour obtained is approximately proportional to the acidity, and may serve as a guide to the requirements of such soils for lime of chalk. The reason for using an alcoholic solution is that the dissociation of the ferric thiocyanate into colourless ions, which would occur in aqueous solution, is prevented.

If the soil is reasonably dry this test may be used in the field.

Lime Requirement. (Hutchinson and McLennan's Method.) An acid soil, when treated with a base such as calcium hydroxide or calcium bicarbonate, absorbs some calcium from the solution, and the consequent reduction of the alkalinity of the solution can be measured. This is the basis of the Hutchinson and McLennan method for determining the so-called " lime requirement " of soils.

Place 20 gm. of the soil in a bottle of 500-1000 ml. capacity, together with 200 ml. of approximately 0·02N. solution of calcium bicarbonate—that is, 100 ml. should neutralise between 19 and 21 ml. of 0·1N. acid. The air in the bottle is displaced by a current of carbon dioxide to avoid possible precipitation of calcium carbonate during the period of the determination. The bottle is stoppered, and then placed in the shaking machine for three hours, after which time the solution is filtered and 50 or 100 ml. of the filtrate is titrated with 0·1N. acid, using screened methyl orange (see appendix) as indicator. The difference in strength of this filtrate and that of the initial solution represents the amount of calcium carbonate absorbed, which can be expressed either as a percentage of the air-dry soil or as tons per acre.

Example.

Weight of soil = 20 gm. Volume of bicarbonate = 200 ml.

50 ml. of $Ca(HCO_3)_2$ (initial solution) = 10·50 ml. of 0·1N. acid.

50 ml. of $Ca(HCO_3)_2$ (from soil filtrate) = 8·25 ml. of 0·1N. acid.

Difference = 2·25 ml.

For the 200 ml. of solution the difference would be 9·0 ml.

From the equation $CaCO_3 + H_2SO_4 = CaSO_4 + H_2O + CO_2$,

1 ml. of 0·1N. acid = 0·005 gm. of $CaCO_3$.

$\therefore$ 9·0 ml. of 0·1N. acid = (0·005 × 9·0) gm. of $CaCO_3$.

Percentage = $0{\cdot}005 \times 9{\cdot}0 \times \frac{100}{20} = 0{\cdot}225$.

Assuming that one acre of air-dry soil 9″ deep has a weight of 1000 tons :

then tons of $CaCO_3$ required per acre = $0{\cdot}225 \times \frac{1000}{100}$

= 2·25.

(Using the above quantities of soil and solution the difference in the titration figures = tons per acre $CaCO_3$.)

Exchangeable or Replaceable Bases or Cations. To obtain a complete picture of base exchange in soils, the following values should be known: (1) the individual exchangeable bases, (2) the total exchangeable bases, (3) the exchangeable hydrogen, (4) the total cation exchange capacity and (5) the degree of or percentage base saturation (100 S/T) where T is the total quantity of cations a soil can absorb or (4) and S is the actual quantity of metallic cations present or (2).

Of the individual exchangeable bases, calcium is the most important and present in largest amount and in most cases is the only one that need be determined. In order to calculate the degree of saturation the amounts of cations are bext expressed as milligram equivalents[1] per 100 gm. of soil. If this is done the sum of (2) and (3) is equal to (4) or if (4) and (2) are determined (3) can be obtained by difference and thus it is only necessary to determine any two of the values (2), (3), or (4) in order to calculate (5).

[1] 1 litre of any normal acid = 1 gm. equivalent of any base, therefore 1 ml. of any normal acid = 1 mgm. equivalent (mgm.e.) of any base. When standard acids are used to determine exchangeable bases, the mgm. equivalents are easily found from the volumes used in the titrations, *e.g.* if 100 gm. of a soil contains bases equivalent to 40 ml. of normal acid, there are 40 mgm. equivalents per cent. present.

Exchangeable or Replaceable Calcium. The method described follows closely the original method proposed by Hissink in which the soil is leached with normal ammonium chloride solution.

In Soils where Calcium Carbonate is absent. To 25 gm. of the air-dry fine earth in a beaker, add 100 ml. of a normal solution of ammonium chloride. Place in a water bath at 80° C., or heat on a sand bath to 80° C., and leave for one hour, stirring at intervals. Allow to stand overnight and filter through a folded filter paper, collecting the filtrate in a litre flask. Transfer the soil to the filter paper with a jet of the normal ammonium chloride solution. Allow to drain through and continue to leach the soil on the filter with successive small portions of the salt solution, allowing it to drain between successive additions, until one litre of filtrate is collected.

Concentrate 500 ml. of the filtrate to about 200 ml., bring to the boil and while boiling add excess of solid ammonium oxalate. Continue boiling for a minute and leave the precipitate of calcium oxalate to settle out over night. Filter, pour off the supernatant liquid, and wash the precipitate by decantation four times with hot water. Then transfer the precipitate to the filter with a jet of hot water, and wash four times with hot water on the filter. Transfer the filter paper and precipitate to the beaker used for the precipitation, add about 100 ml. of distilled water and about 25 ml. of dilute sulphuric acid. Warm the contents of the beaker to about 70° C. and titrate with 0·1N. solution of potassium permanganate.

1 ml. of 0·1N. $KMnO_4 = 0{\cdot}0028$ gm. of CaO.

In Soils where Calcium Carbonate is present. The experiment is similar to the above, but normal sodium chloride is used instead of normal ammonium chloride, and two successive litres of the extract are collected. The amount of calcium present in each litre is determined as before, but the calcium content of the second litre is subtracted from that of the first litre to obtain the exchangeable calcium. This is done because the salt solution has a slight solvent action on the calcium carbonate of the soil, which is proportional to the volume of solution used, while all of the exchangeable calcium is present in the first litre. When ammonium chloride is used to leach soils containing calcium carbonate, it is found that the amount of calcium carbonate dissolved decreases as the leaching proceeds.

Total Exchangeable Bases or Metal Cations. The method

described is Schofield's rapid method which gives the approximate amounts of metal cations present. It is based on the neutralisation of a 0·05N. solution of hydrochloric acid by the exchangeable bases present in the soil, and to obtain reliable results a large excess of the acid must be present.

Weigh an amount of soil (1–5 gm.) into a flask, add 100 ml. of 0·05N. hydrochloric acid, stopper firmly and shake at intervals for four hours. The amount of soil to take will vary with the texture, more being required for a sand or light loam than if the soil is a heavy loam or clay, but not more than sufficient to neutralise about one-fifth of the acid should be taken. Filter through a dry filter paper, reject the first few ml. of the filtrate and collect the remainder in a dry vessel. Pipette a 25 ml. aliquot into a conical flask, add bromo-thymol blue as indicator and titrate with standard limewater. If the colour fades near the end point, add a few more drops of indicator. Titrate 25 ml. of 0·05N. hydrochloric acid with the limewater to obtain the strength of the latter. The total exchangeable bases (S) in mgm.e. per cent. is given by the formula,

$$S = (B - T) \times N \times \frac{100}{25} \times \frac{100}{W}$$

where B = Ml. of limewater used in titrating 25 ml. of 0·05N. HCl,
T = Ml. of limewater used in titrating 25 ml. of soil extract,
N = Normality of limewater,
W = Weight in gm. of soil taken.

N.B.—If calcium carbonate is present in the soil, express its amount in mgm. equivalents per cent., and deduct its value from the figure for total exchangeable bases.

Exchangeable Hydrogen. The method given below, due to Bradfield and Allison, is based on the fact that an acid soil absorbs ammonia from a weak ammoniacal solution which is buffered with ammonium chloride, the reduction in the alkalinity of such a solution being equivalent to the exchangeable hydrogen.

Weigh 2 gm. of air-dry soil into a 250 ml. conical flask and add 100 ml. of a buffer solution which is exactly normal in ammonium chloride and centinormal in ammonia. This solution has a *p*H value of 7·4 and is fairly well buffered at this point. Cork the flask

and shake at intervals for one hour. Allow the suspension to settle, filter through a dry filter paper, reject the first few ml. of the filtrate and collect the remainder in a dry vessel. Pipette 50 ml. of this liquid into a conical flask, add methyl red as indicator and titrate with N./100 hydrochloric acid. If 50 ml. of the soil extract required x ml. of N./100 HCl, the exchangeable hydrogen in mgm.e. per cent. is given by

$$(50 - x) \times \tfrac{100}{50} \times \tfrac{100}{2} \times \tfrac{1}{100} = (50 - x).$$

Total Cation Exchange Capacity (T). This is the sum of the exchangeable hydrogen and the exchangeable bases or metal cations, both being expressed in mgm. equivalents per cent.

Degree of or Percentage Saturation. Calculate this from the formula 100 S/T. Generally speaking there is little response from the application of lime or chalk to agricultural soils, unless the degree of saturation is less than 40 to 50 per cent.

When acid soils are examined by the methods already given, the values obtained are generally related, and in soils of the same type and texture generally vary in the same way. For any particular type of soil arbitrary standards can be established to ascertain whether acid soils require liming, and if so, to calculate the amounts to be used in the field.

MECHANICAL ANALYSIS

The object of mechanical analysis is to obtain information about the size of the ultimate particles of which the soil is composed, and to determine the proportions of various graded fractions. The compound particles are, therefore, broken down with hydrochloric acid and afterwards with ammonia, while hydrogen peroxide is used to destroy the organic matter.

The soil is graded into specific fractions which contain particles within the following limits of size :

Coarse sand	(2-0·2 mm.).
Fine sand	(0·2-0·02 mm.).
Silt	(0·02-0·002 mm.).
Clay	(less than 0·002 mm.).

The coarse sand fraction is separated by a sieve. It is impracticable to use a sieve for the smaller particles, for which

indirect methods have to be adopted, depending on the time taken for the particles to fall through a column of water of given height. Silt and clay are determined in the suspension from which the coarse sand has been removed, by the pipette sampling method, while after removing the silt and clay the fine sand is obtained by sedimentation. In the treatment of the soil with hydrogen peroxide and hydrochloric acid, some of the soil constituents are dissolved—these are usually determined and reported as "loss by solution". In addition, the mechanical analysis usually includes the determinations of the moisture and the carbonates present in the air-dry soil.

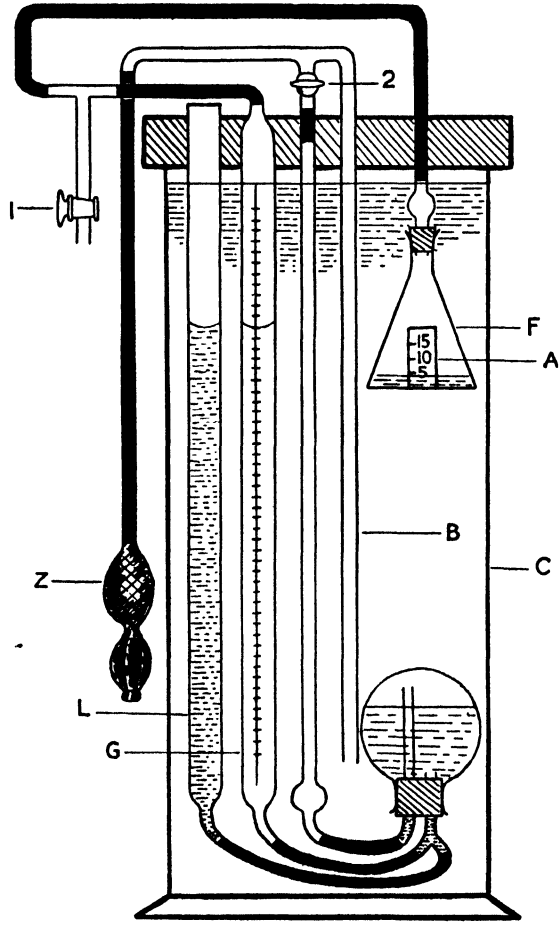


FIG. 2.—Collins' calcimeter.

Determination of Moisture. Weigh 10 gm. of the air-dry fine earth in a wide weighing bottle of known weight, and heat for 24 hours at 105° C.; then cool in a desiccator and weigh. The loss in weight represents the moisture.

Heavy soils and those containing an appreciable amount of organic matter should be replaced in the oven for a further period of 24 hours, and then re-weighed.

Determination of Carbonates. This is most conveniently carried out in the Collins' calcimeter. The diagram shows the essential parts of the apparatus, which is enclosed in a screen of stout copper gauze to protect the parts from breakage.

The apparatus consists of a water jacket in which the various working parts are enclosed, and is provided with a measuring tube graduated from 0 to 50 in tenths of a ml., with an enamelled back for easy reading, and a levelling tube. The level is adjusted by means of a special air flask and bellows, the latter being also used for agitating the water to preserve a uniform temperature.

About 25 gm. of the air-dry fine earth should be ground as finely as possible, and well mixed before a portion is weighed for the determination. The amount of soil required to give reliable results will depend on the carbonate content.

Weigh out the fine soil and place in flask *F* and measure 10 ml. of dilute hydrochloric acid (1 to 3) in tube *A*. Open taps 1 and 2 and close the flask with the rubber stopper; plunge under water in *C*, and keep there by means of the hook. Close tap 2 and blow air through tube *B* in order to stir up the water in the glass cylinder *C* and thus obtain a uniform temperature. Open tap 2, gently squeeze bulb *Z* until the water in the graduated tube *G* stands at zero, and now close tap 1 with the other hand. Release bulb *Z* when the water level in tube *L* descends. Remove flask *F* from the water and tilt the tube *A* containing the acid so that the acid comes in contact with the soil. Shake the flask vigorously for about a minute, and then return the flask to the water and leave for a minute or two to attain the temperature of the water. Close tap 2 after adjusting the level by squeezing the bulb *Z*, so that the water in the graduated tube and in the levelling tube is at the same level. Read the volume of the gas and the temperature on the thermometer which is immersed in the water jacket and also observe the barometric pressure in mm.

The calculation of the result is carried out by means of a special

slide rule. The reading of the barometer is set opposite the reading of the thermometer; then opposite the reading of the number of ml. of acid used will be found the number of mgm. of calcium carbonate which is equivalent to 100 ml. of gas measured on the graduated tube. From this figure and the weight of soil used, the percentage of calcium carbonate in the soil is calculated.

The slide rule takes into account the solubility of the carbon dioxide in water, and also the vapour pressure of the water.

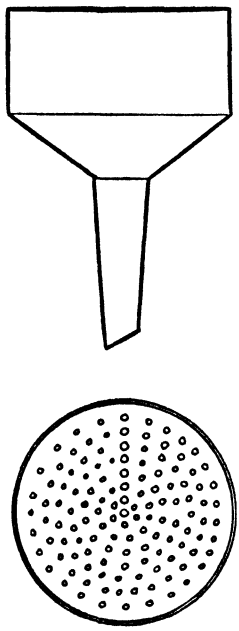


FIG. 3. — Buchner funnel for filtration by suction showing perforated plate.

Grading into Fractions. Two distinct operations are involved: (*a*) dispersion and (*b*) the actual mechanical analysis.

Method of Dispersion. Weigh out 20 gm. of the air-dry fine earth into a tall 600 ml. beaker, add about 60 ml. of 6 per cent. (20 vol.) hydrogen peroxide, and place the beaker in a boiling water bath or on a hot plate. Stir well and note that a vigorous reaction with the humified organic matter soon takes place, and watch the contents of the beaker to avoid frothing over. After the reaction has subsided, make a further addition of hydrogen peroxide if necessary and again heat the beaker. There is usually no great frothing at this stage, unless the soil contains much organic matter, in which case further additions of hydrogen peroxide may be necessary. Allow to cool, and add enough water and hydrochloric acid to give 150 to 200 ml. of 0·2N. acid, allowance being made for the acid required to decompose the carbonates in the soil. Allow the beaker to stand for about an hour (or leave overnight), and frequently stir the contents; then filter, using an 18 cm. hard filter paper, and if available use a filter pump. At first the filtrate may be turbid, but by pouring it back on the filter a clear filtrate can be obtained. Wash the soil on the filter with three separate portions of 100 ml. of distilled water, but do not attempt to wash the soil to the bottom of the filter, since this decreases the rate of filtering and may cause some of the fine material to pass through the filter. After washing is complete, spread out the filter paper on a large clock glass and wash the soil with a jet of hot water on to a wire mesh sieve (IMM No. 70)

with square apertures 0·2 mm. wide, which is held over the mouth of a beaker. When no more soil can be removed, roll the paper into a loose ball, wet it thoroughly and squeeze like a sponge. Repeat this process until no more turbid liquid can be obtained.

Determination of Loss by Solution. The filtrate, after the peroxide-hydrochloric acid treatment, contains, in addition to calcium from calcium carbonate, mixed sesquioxides (oxides of aluminium and iron), and a small quantity of silica which usually amounts to about 2 to 3 per cent. of the total weight of the soil. To determine the dissolved sesquioxides and silica, bring the filtrate to the boil and add ammonium chloride and ammonia in excess. Filter off the precipitate, wash with hot water, dry in the steam oven and gently ignite. The weight of material obtained expressed as a percentage is reported as " loss by solution ".

Determination of Coarse Sand. Gently rub the material on the sieve with a rubber pestle under a jet of water until no more material will pass through. Dry the sieve and residue, transfer the residue to a tared vessel and weigh ; this is the coarse sand fraction.

Mechanical Analysis of the Dispersed Sample by the Pipette Method. Transfer the contents of the beaker containing the soil which passes through the sieve to a litre shaking bottle, and make up to about 500 ml. with distilled water. To this add 50 ml. of 10 per cent. ammonia solution, made by diluting 0·88 sp. gr. ammonia solution with twice its volume of distilled water. Shake the bottle and contents for 24 hours in an end-over-end shaker at a speed of 30 to 40 revolutions per minute. (For sandy soils shaking overnight is sufficient, while very heavy soils may require 40 hours.) After shaking, make up the suspension to 1 litre in a measuring cylinder, and thoroughly shake by repeated inversion of the cylinder for about 1 minute, making certain that any sediment which has settled to the bottom is thoroughly suspended in the liquid. The suspension in the cylinder is equivalent to a 2 per cent. concentration of the original soil. The sampling is done by means of a 20 ml. pipette which is fixed in a cork, so that when the cork rests on the top of the cylinder the point of the pipette is at the required distance below the surface of the suspension.

Determination of Clay and Silt. The first sampling is made at a depth of 10 cm., when the suspension has been allowed to stand for 4 minutes 48 seconds. Mark the pipette so that when in position the point is 10 cm. below the level of the suspension. Shake the

suspension for 1 minute as above, lower the pipette with top closed into the suspension (start this operation about 20 seconds before the time is up), and withdraw 20 ml. of the suspension, taking care to avoid too rapid ingress of the liquid with consequent eddying of the suspension. Deliver the contents of the pipette into a suitable weighed dish (the 72 mm. diameter flat bottom dish commonly used for the determination of total solids in milk is convenient). Evaporate the contents to dryness on a water bath, dry at 105° C., cool in desiccator and weigh. The weight of material multiplied by 5 gives the percentage in the suspension at the point sampled; let this be x. Then the original concentration being 2 per cent., the percentage of material is given by $\frac{100x}{2}$. This calculation reduces in practice to dividing the number of milligrams of the oven dry material by 4. The material obtained in this way represents the sum of the clay and silt fractions.

FIG. 4.—Special pipette for mechanical analysis of soils.

N.B.—A special pipette with or without special device for raising or lowering the pipette may be used. This pipette has a long stem below the bulb, thus obviating the necessity for wide-mouthed cylinders, and has a two-way tap above the bulb. If this pipette is used, lower into the suspension to the desired depth with the tap closed. At the sampling time, give the tap a quarter turn and withdraw the suspension until it fills the pipette to a little above the bore of the tap. Close the tap with another quarter turn andwith draw the pipette. Give the tap another quarter turn when 20 ml. of the suspension is discharged. Return the suspension above the tap to the cylinder.

Determination of Clay. Shake the suspension again for 1 minute, allow to stand for 8 hours and sample as before, but at a depth of 10 cm. below the new surface level. Deliver the suspension into a weighed dish as before, evaporate to dryness, dry at 105° C., cool and weigh. The material obtained is the clay; calculate the percentage as above.

If the above sampling time is inconvenient, either of the following may be used :

7 hours at a depth of 8·75 cm.
10 ,, ,, ,, 12·5 cm.

Determination of Silt. Subtract the percentage of clay from the percentage of the sum of the clay and silt.

Determination of Fine Sand. After the clay sampling, pour away the bulk of the supernatant liquid, transfer the sediment in the cylinder to a 400 ml. beaker and make up with water to a height of 10 cm. Stir the contents thoroughly, allow to settle for 4 minutes 48 seconds and pour away the turbid suspension. Fill up the beaker again to the mark with water and repeat the process until the liquid is no longer turbid at the end of the period. The residue is the fine sand. Transfer this to a weighed dish, dry and weigh as above.

Statement of Results. These should all be given as percentages of the air-dry soil, and include the following :

(1) Coarse sand, remaining on the 0·2 mm. sieve.
(2) Fine sand obtained by sedimentation.
(3) Silt } obtained by pipette sampling.
(4) Clay }
(5) Moisture in air-dry soil.
(6) Carbonates expressed as calcium carbonate.
(7) Loss by solution in peroxide-HCl treatment.
(8) Difference (organic matter removed by hydrogen peroxide and errors of the experiment).

Item 8 is the difference between 100 and the sum of the other seven items. The errors in working with oven-dry material are mainly positive and may occasionally, in soils of low organic content, outweigh the organic matter dissolved by hydrogen peroxide and acid ; in these cases the total of the first seven items will exceed 100.

CHEMICAL ANALYSIS

The chemical analysis of a soil is usually carried out to obtain information regarding the amount of plant food present. The following are the more important determinations which may be

made, while a number of others, *e.g.* chlorides, are carried out in special circumstances : (1) Organic matter, (2) total nitrogen, (3) nitrates, (4) ammonia, (5) inorganic constituents soluble in hydrochloric acid, of which the more important are potassium and phosphate, (6) " available " potassium and phosphate, (7) exchangeable potassium and calcium, and (8) calcium carbonate. The determinations of exchangeable calcium and of calcium carbonate are described on pages 17 and 21 respectively.

Determination of Organic Matter (Loss on Ignition). There is no simple and accurate method for determining the amount of organic matter in a soil. The loss on ignition includes the organic matter and the combined water in the soil colloids, but in the case of soils of similar type the figures so obtained are a good guide to the relative amounts of organic matter present.

Weigh out 10 gm. of air-dry soil, and determine the moisture content as previously described (if this is known omit the determination). Heat the residue to a bright red heat (650° C. to 900° C.), starting with a low flame, which is gradually increased, and finally place in a muffle furnace for 15 to 20 minutes. Cool and weigh. If the soil contains carbonate, carbon dioxide may be lost during the ignition. To correct for this, moisten the residue after cooling with ammonium carbonate solution, heat gently to drive off the excess, cool and weigh. From the loss in weight, calculate the percentage loss on ignition and, if necessary, subtract the percentage of moisture from the sum of the moisture and loss on ignition.

Determination of Organic Matter (from Carbon Content). A number of methods are available for determining the carbon content of soils. It is generally assumed that soil organic matter contains 58 per cent. of carbon, therefore the percentage of organic carbon in a soil must be multiplied by 1·724 to obtain the percentage or organic matter.

The method described is due to Walkley and Black and consists in digesting the soil with potassium dichromate (chromic acid) and sulphuric acid. The excess of chromic acid not reduced by the organic matter is determined by titration with

standard ferrous sulphate. Each ml. of normal potassium dichromate used is equivalent to 3 mgm. of carbon.

It is found, however, that the percentage of carbon determined in this way is less than that determined by the dry combustion process, and varies from about 75 to 80 per cent., with an average of about 77 per cent., of the latter. A correction factor is thus used to arrivė at the approximate percentage of carbon, *viz.* 100/77 and thus 1 ml. of N. potassium dichromate $=3 \times 100/77 = 3{\cdot}9$ mgm. of carbon.

For the determination the soil should be ground to pass a 0·5 mm. sieve. Weigh the soil (1 to 10 gm. depending on the amount of carbon present) and transfer to a 500 ml. conical flask. By means of a pipette add 10 ml. of N. potassium dichromate, and with a measuring cylinder add 20 ml. of concentrated sulphuric acid. Shake for one minute and place the flask on an asbestos mat for about 30 minutes. Add about 200 ml. of water, 10 ml. of phosphoric acid (85 per cent.) and 1 ml. of diphenylamine indicator solution (made by dissolving 0·5 gm. of diphenylamine in 20 ml. of water and adding 100 ml. of concentrated sulphuric acid). Titrate with 0·5N. ferrous sulphate, which should be freshly prepared and made by dissolving 139 gm. of the salt in water, adding 15 ml. of concentrated sulphuric acid and diluting to 1 litre. The ferrous sulphate solution is run in slowly until the colour suddenly changes from blue to green. If more than 8 ml. of N. potassium dichromate is reduced during the digestion, repeat the determination using a smaller quantity of soil. Finally titrate 10 ml. of N. potassium dichromate with 0·5N. ferrous sulphate to standardise the latter using the same quantities of reagents as described above. If x is the ml. of N. potassium dichromate reduced, and W the weight of soil taken, the percentage of organic carbon is given by

$$x \times 0{\cdot}0039 \times 100/W.$$

Notes. (1) With some soil extracts when the 1 ml. of diphenylamine indicator is added the colour changes to green immediately. In such cases another 1 or 2 ml. of indicator should be added and the titration continued as usual.

(2) Soils containing appreciable amounts of chloride give high results as chlorides reduce dichromate solution. In such cases if 25 gm. of silver sulphate per litre is dissolved in the concentrated

sulphuric acid used in the determination, this is usually sufficient to precipitate the chlorides and so prevent their oxidation by the chromic acid.

Determination of Total Nitrogen (Kjeldahl's Method). This determination is often carried out, although a knowledge of the total nitrogen present in a soil is of limited value as a criterion of the nitrogen available to plants. Much work, however, has been done on the carbon-nitrogen ratio in soils and the nitrogen value is needed for this purpose. The method described below gives a measure of the nitrogen in all of the nitrogenous compounds present in a soil, with the exception of nitrate and nitrite nitrogen; the latter, however, rarely account for more than one per cent. of the total nitrogen.

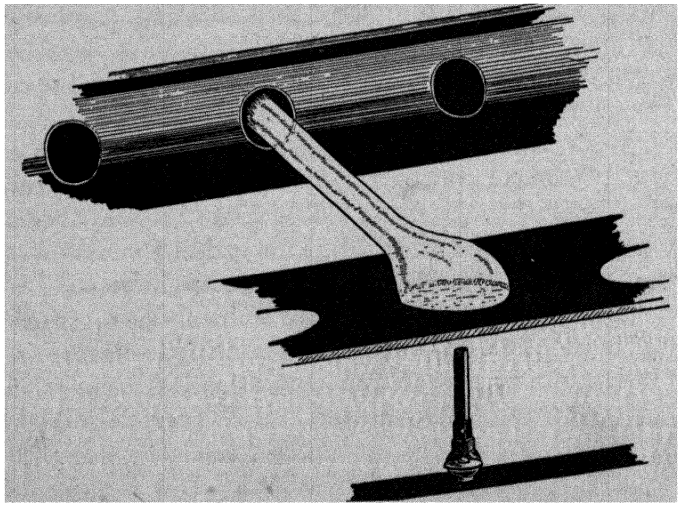


FIG. 5—Kjeldahl digestion flask and fume chamber.

Take about 25 gm. of the air-dry fine earth and grind up as finely as possible in a mortar. Well mix the sample, and weigh out 5 to 10 gm. of the soil into a Kjeldahl flask. Add 10 gm. of potassium sulphate, a crystal of copper sulphate, and finally 20 to 30 ml. of pure concentrated sulphuric acid, revolving the flask as the acid is run in to wash down any soil which may be present in the neck. Heat the flask very gently over a small flame in a fume chamber, and watch for signs of excessive frothing. When the frothing has subsided and the flask is full of white fumes, raise the flame. Heat with occasional shaking until all the carbon has been oxidised and

the contents of the flask are colourless or pale straw in colour. Continue heating for another two hours after this stage is reached, and then allow to cool. Dilute the contents with water and transfer to a 750 ml. conical flask of a distillation apparatus, but leave as much as possible of the insoluble residue in the Kjeldahl flask. Wash out the latter four or five times with water so that the final volume in the distillation flask is about 300 ml.

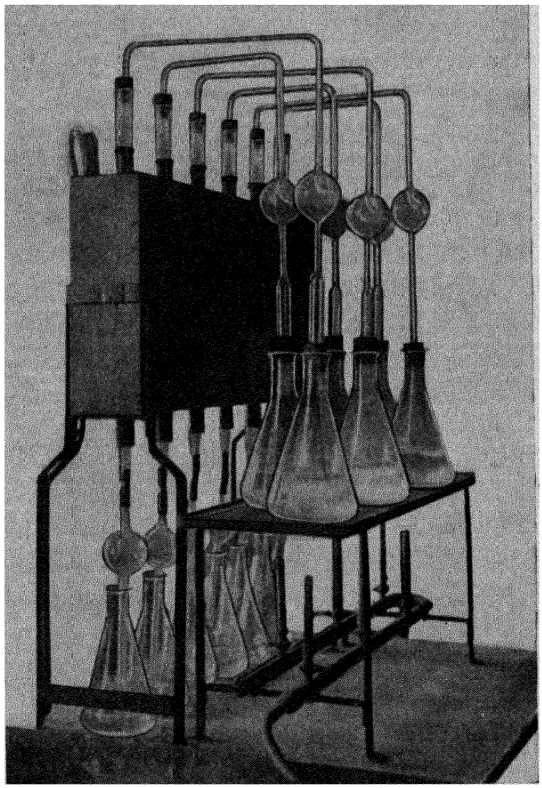


FIG. 6.—Kjeldahl distillation apparatus.

Accurately measure 25 ml. of 0·1N. sulphuric acid into the receiving flask of the distillation apparatus, add a few drops of methyl red or 1 ml. of bromo-cresol green as indicator, and place the flask so that the end of the delivery tube of the apparatus is below the surface of the acid. Add sufficient concentrated sodium hydroxide solution (40 per cent.) to the distillation flask to make the contents alkaline (if 20 ml. of concentrated sulphuric acid was used in the digestion 90 ml. of 40 per cent. sodium hydroxide is sufficient).

When adding the sodium hydroxide pour it carefully down the side of the flask, so that it forms a heavy layer at the bottom, with the acid layer above which prevents any escape of ammonia before the flask can be connected up. Add a very small quantity (0·03-0·04 gm.) of powdered Devarda's alloy, connect the flask to an efficient splash head and the latter to the condenser of the apparatus. Shake the flask so that the contents are well mixed, and distil until about one-third of the contents passes over into the acid of the receiving flask. Remove the end of the delivery tube from the contents of the receiving flask, carry on the distillation for a few minutes longer, and finally wash down the lip of the tube with distilled water. Titrate the excess acid in the flask with 0·1N. sodium hydroxide. From the volume of acid neutralised by the ammonia, calculate the percentage of nitrogen in the soil. 1 ml. of 0·1N. acid = 0·0014 gm. of nitrogen.

Notes on the Method. (1) It is generally desirable that a blank determination should be carried out on about one gm. of sugar.

(2) For heavy clay soils and subsoils, the procedure should be modified as follows. To the soil in the Kjeldahl flask add 10 ml. of water, shake and allow to stand for half an hour. Then proceed as described above.

(3) The potassium sulphate is added to raise the boiling-point of the acid, and so increase the rate of oxidation of the organic matter. The copper sulphate catalyses and greatly speeds up the reaction. Selenium powder may be used instead of the copper sulphate.

(4) During the digestion, the sulphuric acid abstracts from the organic matter the elements of water ; the sulphur dioxide produced by the action of the residual carbon on the sulphuric acid reduces the nitrogenous compounds present. The final result is that all of the nitrogen is changed into ammonia, which is at once fixed by the acid as ammonium sulphate.

(5) The Devarda's alloy is added to prevent bumping during the distillation. It reacts with the alkali to give a stream of hydrogen, which keeps the contents of the flask in continual motion.

(6) If the end point of the titration is not sharp, boil the solution to expel carbon dioxide, cool and complete the titration.

(7) From the equation :

$$2NH_3 + H_2SO_4 = (NH_4)_2SO_4,$$

1000 ml. of N. acid = 17 gm. of NH_3 or 14 gm. of N.

$\therefore$ 1 ml. of 0·1N. acid = 0·0014 gm. N.

Determination of Nitrates. Nitrates fluctuate enormously in amount in the same soil at different times of the year and also from soil to soil. They are easily soluble, and in their determination water only is necessary for their extraction from the soil. The nitrates in the extract are then reduced to ammonia and at the same time traces of nitrites if present will also be reduced.

Obtain a sample of soil taken direct from the field, and dry at once at 55° C. in order to prevent nitrification. Weigh out from 200 to 500 gm. of the dried soil, transfer to a Buchner funnel fitted with a hardened filter paper and attach the funnel to a filter pump. Pour on sufficient hot distilled water to cover the soil. After a few minutes turn on the pump and continue to leach the soil with successive quantities of distilled water, until about 500 ml. of filtrate is obtained. Make up to 500 ml. with distilled water in a graduated flask and shake well. Pipette 200 ml. into a 750 ml. conical flask, add 10 ml. of 8 per cent. sodium hydroxide solution and 10 ml. of 3 per cent. potassium permanganate solution, and cover the flask with an inverted crucible lid. Boil the contents so that the volume is reduced to about 75 ml., and keep just at the boil for about two hours. If the permanganate is completely decolourised, add a little more until no appreciable change occurs in half an hour. The permanganate oxidises any organic matter present in the extract. Dilute the contents to about 300 ml., add 5 ml. of alcohol to destroy the excess potassium permanganate, 20 ml. of 40 per cent. sodium hydroxide and 3 gm. of powdered Devarda's alloy. Connect the flask to an efficient splash head and condenser of a distillation apparatus as in the Kjeldahl method for nitrogen. Place 25 ml. of 0·02N. sulphuric acid in the receiving flask and add 1 ml. of bromocresol green as indicator. Allow the reducing action to go on in the cold for a few minutes, and then distil until the volume is reduced to about 50 ml. The nascent hydrogen produced by the action of the Devarda's alloy on the alkali reduces the nitrates of the extract to ammonia. Titrate the excess acid with 0·02N. sodium hydroxide solution and from the number of ml. of acid used calculate the result as parts of nitrogen per million of dry soil.

1 ml. of 0·02N. acid = 0·00028 gm. nitrogen.

Determination of Ammonia. The amount of ammonia in mineral soils is usually very small, but in acid peat soils, some

pasture soils, heavily dunged soils and in soils after recent applications of ammoniacal fertilisers the amounts may be much higher. The ammonia is retained in the exchangeable form and the method described is based on leaching the soil with sodium chloride, thus replacing the ammonium ions by sodium ions.

Weigh 25 gm. of dry soil (dried quickly as for determination of nitrates) into a beaker, add 100 ml. of normal sodium chloride solution, stir well and leave to stand for half an hour. Pour the supernatant liquid through a large filter paper and collect the filtrate in a litre conical flask. Wash the soil by decantation with another 100 ml. of normal sodium chloride, transfer the soil to the filter and continue to leach the soil with successive quantities of the sodium chloride solution until about 500 ml. of filtrate have been collected. Add 3 to 4 gm. of magnesia, connect the flask to the splash head of a distillation apparatus and pipette into the receiving flask 20 ml. of 0·02N. sulphuric or hydrochloric acid. Heat gently so that about 150 ml. of distillate collects in about half an hour, add 1 ml. of bromo-cresol green as indicator and titrate the excess acid with 0·02N. sodium hydroxide. Calculate the result as parts per million of nitrogen in the dry soil.

Determination of Chlorides. In most soils the chloride content is low and its determination is of little value. However, in soils which have been flooded with sea water and rendered infertile, it is important to know the salt content, as its amount gives a guide to the successful reclamation treatment necessary to bring the soil back to its original fertility.

Weigh 100 gm. of air-dry soil into a flask and add 500 ml. distilled water. Cork and shake for about 15 minutes. Allow the suspension to settle and filter through a dry filter paper, collecting the filtrate in a dry vessel. Pipette an aliquot (50-100 ml.) of the filtrate into a porcelain basin, add a few drops of a 1 per cent. solution of potassium chromate as indicator. Titrate with N./35·5 silver nitrate until a faint tinge of red silver chromate persists. Calculate the percentage of chloride in the original soil as each ml. of N./35·5 silver nitrate used is equivalent to 0·001 gm. chlorine. Thus if x ml. of

silver nitrate were used for a 50 ml. aliquot the percentage of chlorine is

$$x \times 0{\cdot}001 \times 500/50.$$

To convert percentage of chlorine into percentage of salt, multiply by the factor 58·5/35·5.

Mineral Ingredients soluble in Hydrochloric Acid

The results obtained by any of the conventional hydrochloric acid digestion methods are comparable only when the conditions are rigidly observed. The results obtained by different conventional methods are not comparable, since the quantities of the soil constituents passing into solution depend on the ratio of soil to acid, on the strength of the hydrochloric acid used, and on the time of digestion. The method for the preparation of the soil extract given here is a modification of the van Bemmelen-Hissink method, recommended by the Agricultural Education Association in 1931.

Weigh out 20 gm. of air-dry soil which has passed a 2 mm. sieve and place in a tall beaker (500 or 600 ml.). Add 200 ml. of hydrochloric acid of constant boiling-point, cover with a clock glass and boil gently for one hour. Allow to cool slightly, filter, transfer the residue to the filter and wash well with hot water. Add about 25 ml. of concentrated nitric acid to the filtrate, evaporate to dryness, and gently ignite to oxidise the organic matter. Add concentrated hydrochloric acid to the residue, evaporate to dryness and heat for some hours at 120° C. to dehydrate the silica and thus render it insoluble. Extract the residue with dilute hydrochloric acid, filter and well wash the residue with hot water. Make up the filtrate and washings to 500 ml., and label it filtrate *A*.

Determination of Potassium. Evaporate 100 ml. or 200 ml. of filtrate *A* to dryness after adding 0·1 gm. of pure calcium carbonate, if the soil did not effervesce with dilute hydrochloric acid. Ignite the residue to change the chlorides of iron and aluminium into oxides, which are insoluble in water. Extract the residue with hot water, filter and well wash the insoluble residue with hot water. (Retain filter paper and insoluble material for phosphate determination.) Bring the filtrate to the boil, add 5 ml. of a boiling 2 per cent. solution of barium chloride to precipitate any sulphate present. Now add solid ammonium carbonate, and while boiling, add a little solid

ammonium oxalate. In this way the excess barium and any calcium and magnesium present are precipitated, leaving in solution only ammonium, sodium and potassium salts. Filter off the precipitate and well wash with hot distilled water. Evaporate the filtrate to dryness and gently ignite the residue to volatilise ammonium salts. To the residue add hydrochloric acid, evaporate to dryness, and extract with hot water. Filter, collect the filtrate in a small beaker, add 5 ml. of 20 per cent. perchloric acid (free from chloric acid), and evaporate on a sand-bath until white fumes of perchloric acid are copiously evolved. Add sufficient water to redissolve the perchlorates and a few drops of perchloric acid solution, and again concentrate to the fuming stage. Cool, add 20 ml. of alcohol (95-96%), and break up the crystals with a glass rod. Allow the precipitate to settle and then filter, pouring the clear liquid as completely as possible through a weighed Gooch crucible prepared as below. Wash the precipitate by decantation with two successive portions of 20 ml. of alcohol saturated with potassium perchlorate (at the temperature at which it is used). Transfer the crystals to the Gooch crucible with the aid of a jet of the alcohol perchlorate solution, and finally wash the crystals in the crucible twice with some of the solution. Dry in an oven at 100° C. for 2 hours, cool and weigh the potassium perchlorate ($KClO_4$). As the percentage of potash is calculated as potassium oxide (K_2O), the weight of precipitate must be multiplied by the appropriate factor. Thus $2KClO_4 = K_2O$ and therefore 1 part $KClO_4 = 0{\cdot}3401$ part K_2O.

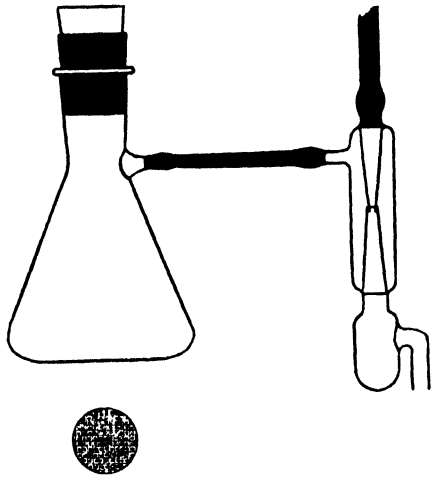


FIG. 7.—Filter pump with Gooch crucible showing perforated disc.

Notes. (1) The $CaCO_3$ is added, since in the absence of sufficient $CaCO_3$ the potassium in the ignited residue is liable to be only partially soluble in water.

(2) The Gooch crucible is prepared with special asbestos which has been previously boiled with acid and alkali and finally well washed with water. Sufficient of the asbestos is added to the

crucible to form a mat of about $\frac{1}{8}$ inch thickness. It is washed first with alcohol (95-96%), then with alcohol saturated with potassium perchlorate and finally dried at 100° C., cooled and weighed.

(3) The final precipitate consists of the perchlorates of sodium and potassium only, produced from the chlorides of these elements during the evaporation. Sodium perchlorate is soluble in alcohol, while potassium perchlorate is almost insoluble; therefore alcohol saturated with potassium perchlorate is used for the washing on account of the slight solubility of the salt.

Determination of Phosphoric Acid. Transfer the filter paper and residue from the potash determination to the original dish, add 50 ml. of 10 per cent. sulphuric acid, and heat on a sand-bath for half an hour. Filter and wash well with hot water. To the filtrate add ammonia until alkaline, reacidify with nitric acid, and add about 100 ml. of ammonium molybdate solution. Warm to 55° C. and keep at this temperature for about ten minutes. Allow to stand for at least two hours, filter and wash the precipitate with a 2 per cent. solution of sodium nitrate until free from acid. Transfer the filter paper with the precipitate to the beaker used for the precipitation, and add a known volume of 0·1N. sodium hydroxide so that the precipitate completely dissolves. Add 2 ml. of phenolphthalein solution as indicator, and titrate the excess alkali with 0·1N. sulphuric acid. Calculate the percentage of phosphoric acid (P_2O_5) in the soil.

1 ml. of 0·1N. alkali = 0·0003004 gm. of P_2O_5.

Notes on the Method. (1) A solution of sodium nitrate is used for washing the precipitate of ammonium phospho-molybdate, as water induces deflocculation of the precipitate, and the latter may then pass through the filter paper.

(2) The equation for the reaction of sodium hydroxide with the ammonium phospho-molybdate is complicated and theoretically $46NaOH = P_2O_5$. This gives a factor 0·0003088 gm. $P_2O_5 = 1$ ml. of 0·1N. alkali. The reaction, however, does not quite conform to the theoretical equation and the factor given above is more accurate under the conditions of the determination.

Determination of Iron and Aluminium. Pipette 100 ml. of filtrate *A* into a beaker, add 10 ml. of concentrated nitric acid and boil for a few minutes. Allow to cool slightly and slowly add ammonia solution (1 to 1) until a slight excess is present. Raise to the boiling-point and then allow the precipitate to settle. Filter, pour off the

supernatant liquid, and wash the precipitate three or four times by decantation with hot water. Transfer the precipitate to the filter and wash with hot water until free from chlorides. (Retain the filtrate for the calcium and magnesium determinations.) Dry the filter paper and precipitate, and then remove the precipitate as completely as possible to a weighed crucible. Ignite the paper in a platinum wire, shake the ash from the wire into the crucible, and finally heat the crucible to a bright red heat for 15 to 20 minutes. Cool and weigh the crucible which contains Fe_2O_3, Al_2O_3 and P_2O_5.

Determination of Iron. Pipette 50 ml. of filtrate *A* into a beaker, precipitate the iron as the hydroxide and wash the precipitate as above. Dissolve the precipitate by pouring dilute sulphuric acid on the filter, collecting the filtrate in a conical flask. Add 5 to 10 gm. of pure zinc (free from iron) to the flask and leave until the nascent hydrogen produced reduces all of the iron to the ferrous state. Test whether the reduction is complete by withdrawing one drop of the liquid on the end of a glass rod and bringing it into contact with one drop of a solution of potassium thiocyanate on a white tile. If any ferric salt still remains a red colour is produced. When the reduction is complete filter off the excess zinc by pouring through a funnel containing a wad of glass wool, wash well with cold water, and titrate the contents immediately with 0·1N. potassium permanganate solution. Express the result as percentage of ferric oxide (Fe_2O_3).

1 ml. of 0·1N. $KMnO_4 = 0{\cdot}0080$ gm. Fe_2O_3.

Determination of Aluminium. From the combined percentage of Fe_2O_3, Al_2O_3 and P_2O_5, subtract the percentages of Fe_2O_3 and P_2O_5 and thus obtain the aluminium as percentage of aluminium oxide (Al_2O_3). If titanium is present in the soil the percentage of Al_2O_3 obtained by difference will be unduly high, because this is precipitated with ammonia and will be present as titanium oxide (TiO_2) in the weighed residue of the iron and aluminium determination.

Determination of Calcium. Concentrate the filtrate from the iron and aluminium determination to about 100 to 150 ml. While boiling add excess of solid ammonium oxalate and keep boiling for a minute. Allow to stand for four hours, filter and wash the precipitate twice by decantation. Dissolve the precipitate in the beaker in dilute hydrochloric acid and add sufficient water to make the volume about 100 ml. Add ammonia until slightly alkaline and reprecipitate the calcium as above. This double precipitation is

necessary because the first precipitate is usually contaminated with magnesium. Allow to stand for four hours, filter and wash the precipitate (p. 17), using the same filter paper as in the first filtration. Complete the determination of the calcium as previously described (p. 17), and express the result as percentage of calcium oxide.

Determination of Magnesium. Boil the filtrate from the calcium determination until the volume is about 100 ml. Allow to cool, and add 6 to 7 ml. of concentrated ammonia, making a final concentration of about 2 per cent. of ammonia. Slowly add excess of a solution of sodium phosphate (Na_2HPO_4), vigorously stirring the whole time with a rubber-tipped glass rod. Allow to stand overnight, filter and wash the precipitate of magnesium ammonium phosphate with a 2 per cent. ammonia solution until free from chlorides. Dry the filter paper and precipitate in the steam oven, transfer as much as possible of the precipitate to a weighed crucible and ignite the filter paper in a platinum wire. Add the ash to the crucible, and heat the latter first over a bunsen flame and finally in a muffle furnace at a bright red heat for 20 minutes. In the heating, water and ammonia are driven off, and the residue in the crucible is magnesium pyrophosphate ($Mg_2P_2O_7$). Cool and weigh the crucible, and from the weight of $Mg_2P_2O_7$ obtain the weight of magnesium oxide (MgO) from the factor,

$$1 \text{ part of } Mg_2P_2O_7 = 0{\cdot}3621 \text{ part of MgO.}$$

Calculate the result as percentage of magnesium oxide (MgO). The equations in the above reactions are :

$$MgCl_2 + NH_4OH + Na_2HPO_4 = MgNH_4PO_4 + 2NaCl + H_2O.$$
$$2MgNH_4PO_4 = Mg_2P_2O_7 + 2NH_3 + H_2O.$$

Available Potassium and Phosphoric Acid

Many attempts have been made from time to time to obtain information regarding the amounts of potassium and phosphorus compounds present in soils, which are in a form available to plants. It is important to realise that the methods at present in use of extracting " available constituents " from soil are not fundamental, but conventional in character, and that in consequence, the details of each method must be strictly adhered to. Different dilute acids of varying concentrations have been used for extracting the soil, the one generally adopted in this

country being one per cent. citric acid, advocated by Dyer. In spite of imperfections, it is still largely used, and the large accumulation of data now existing from the use of this method warrants its description as given below.

In recent years, the exchangeable bases have come to be regarded as the more probable nutrients of plants, as they represent a natural division and not an arbitrary group of soil constituents. It is reasonable to assume that the available nutrients will be contained in the exchangeable portion, and in the case of potassium the acetic acid method is described for the determination of this element present in the exchangeable form.

The 0·3N. hydrochloric acid method described below hardly differs in principle from other conventional methods and its speed commends it for class work. The determination of phosphate depends on the fact that the phospho-molybdate compound, formed by the interaction of a phosphate solution with ammonium molybdate and sulphuric acid, produces a blue coloration in the presence of a suitable reducing agent such as stannous chloride. The determination of potassium is based on the fact that low concentrations of this element can be precipitated as potassium cobaltinitrite in the presence of excess ethyl alcohol.

The method is essentially a modification of the Hellige-Truog, used in the U.S.A. It is extremely rapid in use if a Hellige colour plate of glass standards, comprising a range of blue colours for phosphate, and of turbidities for potash, is available for matching the soil extracts. Alternatively the blue colours can be matched in a colorimeter, or the colours and turbidities may be determined by a photo-electric absorptiometer. If none of the above apparatus is available, the phosphate extracts can be matched in Nessler cylinders and the potash determined in much larger amounts of soil, by the perchlorate method.

In Germany during the last twenty-five years two methods have been evolved, the Mitscherlich and the Neubauer, both of which depend upon the plant itself for an indication of the amount of available plant nutrients. In the former, oat seeds

are grown in pots of soil under different manurial treatments. At maturity the grain and straw are harvested separately, and the weight of dry matter in each determined. In the other method rye seeds are grown in the soils, but no manurial dressings are given and the seedlings are grown for seventeen days only from the date of sowing. The seedlings are then analysed, and the amounts of potash and phosphoric acid present are found. In both methods the results should be correlated with those obtained under field conditions. The methods, however, because of the time taken and the technique involved, are unsuitable as students' exercises.

Another method which has developed recently consists in adding a specific culture solution to the soil and then inoculating with the mould Aspergillus Niger. When available potash is to be determined ammonium phosphate is added, and when available phosphoric acid is to be determined potassium sulphate is added. The mixture is incubated for four to six days, and the dry weight of the mycelium produced is taken as an indication of the available potash or phosphoric acid in the soil.

Determination of Citric-Soluble Potash and Phosphoric Acid. Weigh out 100 gm. of the air-dry fine earth into a Winchester quart bottle and add 1000 ml. of distilled water in which are dissolved 10 gm. of citric acid. Allow the soil to remain in contact with the solution for seven days, shaking a number of times each day. If a mechanical shaker is available, 24 hours' continuous shaking is sufficient. Filter and collect 500 ml. of the filtrate in which the amounts of potash and phosphoric acid are determined. Evaporate the solution to dryness, and gently ignite the residue until it flakes off the side of the dish. Grind the residue to a powder with a small pestle and ignite again until all of the organic matter is destroyed. Cover the residue with strong hydrochloric acid, evaporate to dryness, and heat for some hours at about 120° C. to render the silica insoluble.

Extract the residue with hot water to dissolve the potassium chloride, and determine the amount present as on p. 34. Then digest the insoluble material left with dilute sulphuric acid, and determine the amount of phosphoric acid present as on p. 35. Express the results as percentages of K_2O and P_2O_5 respectively.

N.B.—If calcium carbonate is present, sufficient citric acid must be added to neutralise it, and for every 1 gm. of calcium carbonate present an extra 1·4 gm. of citric acid is added.

Determination of Exchangeable Potash. Weigh 25 gm. of soil into a beaker, add 200 ml. of 0·5N. acetic acid solution and after stirring several times allow to stand for at least two hours. Filter, pour off the supernatant liquid as completely as possible and collect the filtrate in a litre flask. Transfer the soil to the filter paper and leach with 0·5N. acetic acid until 1 litre of filtrate is obtained. Add about 20 ml. of hydrochloric acid and evaporate to dryness, the acetic acid being removed in this way. Cover the residue with concentrated hydrochloric acid, evaporate to dryness and ignite the residue for several hours to dehydrate the silica. Extract with hot water, filter and determine the amount of potassium present in the filtrate as on page 34, expressing the result as percentage of potash (K_2O).

Extraction with 0.3N. Hydrochloric Acid (for soils with less than 3 per cent. chalk). Weigh 6 gm. of soil into a test-tube, add 14 ml. of 0·3N. hydrochloric acid, cork and shake vigorously for one minute. Filter, using a small filter paper. (For gravimetric determination of potash in the extract, 50 to 100 gm. of soil should be used and the acid added in proportion.)

Phosphate. (Rapid Method.) Pipette 0·5 ml. of the soil extract into a specimen tube (about 4 in. high and 20 mm. diameter), add 7·5 ml. of distilled water and 0·1 ml. of a specially prepared ammonium molybdate in sulphuric acid solution. Mix the contents, add a pinch (about 0·003 gm.) of dry stannous chloride, shake gently and allow to stand for one minute. Compare the blue colour obtained with the Hellige glass standards, the phosphate values of which have been previously determined by comparison with phosphate solutions of known strength, in which the blue colour has been developed under identical conditions.

Phosphate. (Hellige glass standards not available.) Pipette 1 ml. of the soil extract into a 100 ml. Nessler cylinder, dilute to the mark with distilled water and add 0·25 ml. of the molybdate sulphuric acid reagent. Mix the contents, add a little (about 0·006 gm.) dry stannous chloride, shake gently and at the end of one minute compare the blue colour with phosphate standards made at the same time. For the first match, it is best to make up standards corresponding to 10 parts and 5 parts P_2O_5 per 100,000 of soil. If

the colour produced is deeper than that of the higher standard, less of the extract should be taken. For the final matching, standards differing by 1 part, 0·5 part or 0·25 part of P_2O_5 per 100,000, depending upon the concentration to be matched, should be made up. At concentrations of about 10, 5 and 2 parts P_2O_5 per 100,000 of soil, differences of 1, 0·5 and 0·25 parts per 100,000 respectively can be detected.

Potash. (Rapid Method.) Pipette 0·75 ml. of the soil extract into a specimen tube (about 4 in. high and 20 mm. diameter) and add 3·3 ml. of a special acetate-alcohol solution. Fit the closed end of a small ignition tube (about 5 mm. diameter) into a rubber stopper which fits the tube, and introduce 0·01 gm. of sodium cobaltinitrite (Analar) into the ignition tube. Add three drops of distilled water and stir with a glass rod until the solid is *completely* dissolved. Insert the stopper into the tube containing the test solution taking care that no liquid leaves the ignition tube at this stage. Holding the tube obliquely at arm's length, jerk the arm sharply downwards and so discharge the contents of the small tube, with considerable force, into the contents of the larger one. Pause for 10 seconds and finally shake the tube gently to and fro 10 times. Measure the degree of turbidity by placing the tube in the Hellige comparator and noting the extent to which it obscures the black bars of the plate, in comparison with the obscuring effect of the permanent turbidity standards. If no absolute match is obtained, less of the soil extract should be used (*e.g.* if 0·5 ml. is taken, add 0·25 ml. of water to keep the conditions constant), so that a lower glass standard matches the soil extract. The values of the latter in terms of potassium must be ascertained by comparing with solutions of potassium chloride of known strength, in which the potassium has been precipitated under identical conditions.

Potash. (Hellige glass standards not available.) Determine the potash in a suitable aliquot of soil extract (50 to 150 ml.) in exactly the same way as described under the hydrochloric extract of soil on page 34.

In recent years many analyses have been carried out by the above methods on large numbers of soils and the results correlated with field results. In non-calcareous soils (less than 3 per cent. of chalk) the amounts of phosphate extracted by 0·5N. acetic acid and by 0·3N. hydrochloric acid are of the same order

of magnitude and in general the figures are very similar. The citric acid method, however, usually extracts much more phosphate, particularly in acid soils, and this point must be borne in mind when limiting values are fixed below which responses to phosphatic fertilisers may be expected.

The amounts of potash extracted by the three solvents are of the same order of magnitude, the figures for 0·3N. hydrochloric acid being on the average slightly higher than those for 0·5N. acetic acid, and the latter slightly higher than the citric acid figures. In individual soils, however, the results obtained by the three methods may not always be in this order.

CHAPTER II

FERTILISERS AND MANURES

THE large number of fertilisers and manures used in agricultural and horticultural practice are very varied in character. Many contain only one fertilising constituent and are grouped as nitrogenous (inorganic or organic), or phosphatic, or potassic manures. The " mixed " or general manures supplying two or three of the fertilising constituents include potassium nitrate, the guanos, bone manures (usually regarded as mainly phosphatic), farmyard manure, poultry manure, compound manures, and the more recently introduced " concentrated " fertilisers. The different forms of " lime " although not usually added to the soil as direct fertilisers, are included in this section. The exercises given demonstrate the general properties of the manures, and the methods by which the chief manurial constituents are determined.

Sampling for Quantitative Analysis. The proper sampling of manures is of great importance. The student is usually less concerned in the taking of the sample from bulk, than in the sample as received in the laboratory. Generally, however, a much larger sample than is actually needed for the analysis is ground finely and well mixed, and from this the small portion required is weighed out. In the case of substances which lose or gain weight during the grinding, the amount of water in the coarse sample and in the powdered sample respectively is determined, and the results of the powdered sample are then recalculated to the water content of the original coarse substance. Materials such as shoddy should be cut up as finely as possible with scissors; while with farmyard manure or poultry manure, which may be analysed in the fresh state, any large particles, such as undecomposed straw, are cut up, the whole thoroughly mixed and the sample weighed as quickly as possible.

The samples should be kept in dry bottles with air-tight stoppers, to preserve the original composition of the fertiliser.

NITROGENOUS FERTILISERS

These contain nitrate, ammoniacal, cyanamide or organic nitrogen, but the percentage of nitrogen should always be stated as the element nitrogen.

Sulphate of Ammonia. There are two sources of supply, (1) the by-product from gas works and coke ovens, and (2) the synthetic product manufactured from the nitrogen of the atmosphere. The fertiliser usually contains about 21 per cent. of nitrogen, representing a purity of 99 per cent.

To show how Ammonia is obtained from Coal. When coal is heated in a closed vessel it is carbonised, gaseous products (coal gas) and liquid products (gas tar and gas liquor) are obtained and coke is left in the vessel. The gas liquor contains ammonia and ammonium salts.

Place some coal in a hard glass test tube and attach a delivery tube, bent at a right angle. Heat strongly and collect the tarry liquid which distils over. Pour off the upper aqueous layer into a test tube, add a little sodium hydroxide solution and heat. Note that ammonia is given off.

Determination of Free Acid. Weigh 20 gm. of the sample into a beaker, add about 100 ml. of distilled water and stir until dissolved. Add a few drops of methyl red as indicator, and titrate with 0·1N. sodium hydroxide. Calculate the acidity as percentage by weight of sulphuric acid (H_2SO_4).

1 ml. of 0·1N. NaOH = 0·0049 gm. H_2SO_4.

The " neutral " grades of sulphate of ammonia should not contain more than 0·025 per cent. of free acid expressed as sulphuric acid.

Determination of Nitrogen. Accurately weigh about 5 gm. of the sample and transfer to a 500 ml. graduated flask. Dissolve in water, make up to the mark and shake well. Pipette 25 ml. of the solution into the distilling flask of a nitrogen distillation apparatus. Add about 300 ml. of water, 10 ml. of 40 per cent. sodium hydroxide solution and a small amount of Devarda's alloy. Distil into 50 ml. of 0·1N. sulphuric acid, and titrate the excess with 0·1N. sodium hydroxide. From the volume of acid neutralised by the ammonia, calculate the percentage of nitrogen in the sample.

1 ml. of 0·1N. H_2SO_4 = 0·0017 gm. NH_3 = 0·0014 gm. N.

Oxidation of Ammonia to Nitric Acid, Much of the nitric acid used in the manufacture of synthetic nitrogenous fertilisers is obtained by the oxidation of ammonia.

Place some platinised asbestos in a hard glass combustion tube. Attach a Woulffe's bottle containing a strong solution of ammonia to one end and a tube leading into a distilling flask to the other end.

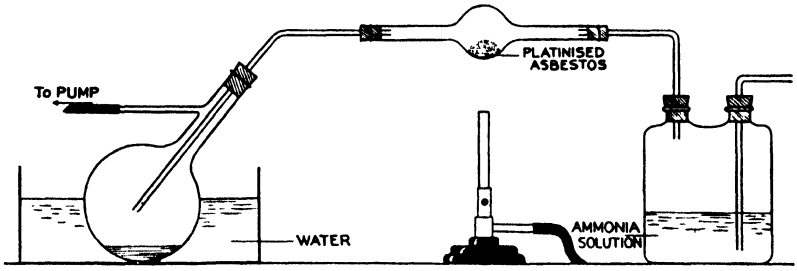


FIG. 8.—Apparatus for oxidation of ammonia.

Attach the side tube of the distilling flask to a suction pump and heat the platinised asbestos.

Note that a liquid collects in the distilling flask, which also becomes filled with brown fumes. Test the liquid to show that it is nitric acid.

$$NH_3 + 2O_2 = HNO_3 + H_2O.$$

Nitrate of Soda. The natural product from Chili has been used for many years, but a synthetic product is now produced. The coarse crystalline form contains about 15·5 per cent. of nitrogen, representing a purity of 94 per cent., while a granular form introduced in recent years contains at least 16 per cent. of nitrogen. Some sodium chloride is present, and the Chilian nitrate usually contains a trace of iodine combined as iodate.

Qualitative Test for Iodate. Dissolve a few gm. of the salt in water in a test tube, acidify with dilute hydrochloric acid, and add a few ml. of a weak solution of sodium sulphite. Test for free iodine by the addition of starch solution. If iodine is found, the presence of iodate is indicated, the iodate being reduced to iodine by the sulphur dioxide from the sodium sulphite.

$$2HIO_3 + 5SO_2 + 4H_2O = 5H_2SO_4 + I_2.$$

Determination of Chlorides. Weigh 5 gm. into a beaker, and add about 100 ml. of distilled water to dissolve the salt. Add a few

Qualitative Test for Ammonia. Dissolve a few gm. of the sample in water and heat with a few ml. of sodium hydroxide solution. Test the vapour for ammonia by means of litmus paper.

Determination of Total Nitrogen. Carry out the determination as described for nitrate nitrogen in nitrate of soda, using the same weight of fertiliser. Owing to its deliquescent nature, it is important that the sample be weighed quickly in a weighing bottle.

Nitro Chalk. This consists of a mixture of approximately 45 per cent. ammonium nitrate and about 50 per cent. of calcium carbonate. It is marketed as a grey granular product with a nitrogen content of 15·5 per cent.

Determination of Ammoniacal Nitrogen. Weigh 5 gm. of the sample into a beaker and dissolve in dilute hydrochloric acid. Transfer the solution to a 500 ml. graduated flask, wash out the beaker with water several times, and then make up to the mark with water.. Shake well and pipette 25 ml. of the solution into the flask of a distillation apparatus. Add water to make the volume about 300 ml., and determine the percentage of ammoniacal nitrogen present as described in the determination of nitrogen in sulphate of ammonia using fragments of pumice instead of Devarda's alloy.

Determination of Total Nitrogen. Proceed as described for nitrate nitrogen in nitrate of soda.

Determination of Nitrate Nitrogen. Subtract the percentage of ammoniacal nitrogen from the percentage of total nitrogen.

Determination of Calcium Carbonate. Weigh 5 gm. of the sample, dissolve in dilute hydrochloric acid and transfer to a 500 ml. graduated flask. Make up to the mark with distilled water, shake well and filter. Pipette 20 ml. of the filtrate into a beaker, make slightly alkaline with ammonia and reacidify with acetic acid. Dilute the volume to about 100 ml. and determine the calcium by the oxalate permanganate method (p. 17). Calculate the result as percentage of calcium carbonate ($CaCO_3$).

1 ml. of 0·1N. $KMnO_4 = 0·005$ gm. $CaCO_3$.

Calcium Cyanamide or Nitrolim. This is a synthetic product made by the combination of nitrogen with calcium carbide. It usually contains 20·6 per cent. of nitrogen, representing a purity of 60 per cent. ; the remainder consists chiefly of carbon and slaked lime with a little oil which has been added to overcome the dustiness of the material.

Qualitative Tests. (*a*) Heat a little of the sample with a dilute solution of sodium hydroxide. Test the vapour for ammonia with litmus and turmeric papers.

(*b*) Shake a few gm. of the sample with water and filter. To the filtrate add silver nitrate solution. Note the yellow precipitate of silver cyanamide.

$$CaCN_2 + 2AgNO_3 = Ag_2CN_2 + Ca(NO_3)_2.$$

(*c*) Fuse a few gm. of the fertiliser with about three times its weight of sodium chloride by heating in a crucible for 10 minutes at a bright red heat. (This converts the cyanamide into cyanide.) Extract the mass with water and filter. Add to the filtrate a few drops of solutions of ferrous sulphate and ferric chloride, and acidify with hydrochloric acid. The presence of cyanide is indicated by the formation of a precipitate or coloration of Prussian blue.

Determination of Total Nitrogen. Accurately weigh about 0·25 gm. of the sample and transfer to a Kjeldahl flask, add 50 ml. of distilled water, 10 gm. of potassium sulphate, a crystal of copper sulphate and 20 ml. of concentrated sulphuric acid. Heat gently for one to two hours until the water has boiled off, and then more strongly for a further two to three hours until digestion is complete. Complete the determination as on p. 29, using 50 ml. of 0·1N. acid in the receiving flask.

Organic Nitrogenous Manures. These include a large number of substances, some of which are much favoured by market gardeners. The following may be mentioned, and if time permits should be analysed for total nitrogen by the Kjeldahl method (p. 28).

Hoof and Horn meal (10 to 15 per cent. nitrogen).

Shoddy (very variable with 3 to 15 per cent. nitrogen).

Dried Blood (12 to 14 per cent. nitrogen).

Soot (3 to 5 per cent. nitrogen).

Waste Cakes, *e.g.* rape-cake and castor meal (3 to 6 per cent. nitrogen).

Determination of Total Nitrogen. Weigh from 2 to 5 gm. of the sample, and use 30 ml. of concentrated sulphuric acid in the digestion. After the digestion dilute the contents and make up to a known volume, *e.g.* 500 ml. or 1000 ml. From this solution pipette an aliquot volume for the distillation.

PHOSPHATIC FERTILISERS

The phosphate may be present as water-soluble or insoluble phosphate, the amount being stated as percentage of phosphoric acid (P_2O_5).

Superphosphate. This is obtained by treating finely ground rock phosphates with sulphuric acid, the phosphate being mainly present as water-soluble mono-calcium phosphate $CaH_4(PO_4)_2$. It is sold with a guarantee of the amount of water-soluble phosphoric acid, which may vary from 14 to 18 per cent.

Preparation of Water-soluble Phosphate from Mineral Phosphate. In the manufacture of superphosphate mineral phosphate is treated with just sufficient sulphuric acid to convert the tricalcic phosphate to soluble monocalcium phosphate.

$$Ca_3(PO_4)_2 + 2H_2SO_4 = CaH_4(PO_4)_2 + 2CaSO_4.$$

Place some ground mineral phosphate in an evaporating dish and add just sufficient strong sulphuric acid to moisten it. Mix well with a glass rod and allow to stand for about an hour. Place some of the mixture in a test tube and shake up with water. Filter and test the filtrate for phosphate with ammonium molybdate solution. The presence of a yellow precipitate shows that the mixture contains water-soluble phosphate.

Qualitative Examination. (*a*) Test a sample of superphosphate for acidity, aluminium, iron, soluble calcium, sulphate and soluble phosphate.

(*b*) Mix a small quantity of superphosphate with some nitrate of soda by rubbing together in a mortar. Heat some of the mixture in a test tube with a few drops of water, when free nitric acid will be formed if the fertiliser contained free acid.

Determination of Water-soluble Phosphoric Acid. Weigh 20 gm. of the sample and transfer to a litre flask, add about 800 ml. of water at room temperature and shake continuously for 30 minutes. Make up to the mark with water, shake well and filter. Pipette 50 ml. of the filtrate into a beaker, add about 20 ml. of concentrated nitric acid and boil. Allow to cool to 70° C. and add approximately 100 ml. of special ammonium molybdate solution (an excess). Warm again to 70° C., allow to cool, and filter through a " folded " filter

paper. Wash the precipitate several times by decantation, and finally on the filter with one per cent. nitric acid solution. Dissolve the precipitate by pouring several portions of cold 2 per cent. ammonia solution on to the filter, using about 100 ml. in all, and collect the filtrate in the beaker used for the precipitation. Add, drop by drop, 15 ml. of magnesia mixture to the solution, with constant stirring. Allow to stand overnight and filter. Wash the precipitate of magnesium ammonium phosphate with 2 per cent. ammonia until free from chlorides. Dry in the oven, ignite (p. 37), and weigh the magnesium pyrophosphate. Calculate the result as phosphoric acid (P_2O_5).

1 part $Mg_2P_2O_7 = 0{\cdot}6378$ part P_2O_5.

Triple Superphosphate. This material containing from 40 to 48 per cent. of water-soluble phosphoric acid was imported from the U.S.A. during the second world war. In its production, liquid phosphoric acid is used instead of sulphuric acid. Apart from its much higher phosphate content, it differs from superphosphate in that it contains very little calcium sulphate.

Determination of Water-soluble Phosphoric Acid. Carry out the determination as described under superphosphate, using 10 gm. instead of 20 gm.

Basic Slag. Basic slag is a by-product obtained in the manufacture of steel from pig-iron. The phosphoric acid probably exists in several combinations, chiefly with calcium and silicon, and is insoluble in water. It is sold with a guaranteed content of phosphoric acid, which varies in different grades and ranges from 10 to 18 per cent. Basic slag contains some free lime, silica, sulphides, carbonates, free iron and magnetic iron oxide. A sample may be examined qualitatively for the presence of these substances.

Determination of Fineness. The value of a slag depends to a large extent on the fineness of division. Under the provisions of the Fertilisers and Feeding Stuffs Act of 1932, the sale of slag must be accompanied by a statement giving the amount of the article which will pass through a prescribed sieve. This sieve

has approximately 100 meshes per linear inch (British Standard Test Sieve, mesh number 100), and 80 per cent. or more should pass through the sieve.

Dry the sample at 100° C., weigh 20 gm. and transfer to the sieve with the lower receiver attached. Shake for ten minutes with occasional tapping of the sides, and then weigh the fine material which has passed through the sieve. Replace the sieve, repeat shaking for another ten minutes, and weigh the sifted material. Repeat this process until not more than 0·2 per cent. is sifted during ten minutes.

Determination of Total Phosphoric Acid. Weigh 5 gm. of the sample into a Kjeldahl flask, add 20 ml. of concentrated sulphuric acid and heat until white fumes appear. Allow to cool, dilute with water and wash out the contents into a 500 ml. flask, make up to the mark, shake well, filter, and pipette 100 ml. of the filtrate into a beaker. Add ammonia until just alkaline, reacidify with nitric acid, and complete the determination as described for superphosphate.

Determination of Citric-soluble Phosphoric Acid. The Fertilisers and Feeding Stuffs Act does not compel the vendor to make any statement regarding the solubility of the phosphoric acid in basic slag. The value of basic slag, however, is closely related to the amount of phosphoric acid which is soluble in citric acid, and in a high grade slag 75 per cent. or more of its phosphoric acid should be soluble (under prescribed conditions) in 2 per cent. citric acid solution.

Weigh 5 gm. of the sample into a stoppered bottle of about 1 litre capacity. Moisten with 5 ml. of alcohol or methylated spirits to lessen the possibility of caking, and add 495 ml. of a solution containing 10 gm. of pure crystallised citric acid. Shake continuously for 30 minutes, filter through a large "folded" filter paper, and determine the phosphoric acid in 50 ml. of the filtrate by the molybdate-magnesia method (p. 49).

Mineral or Ground Rock Phosphate. Mineral or rock phosphate is mined in many parts of the world. It is used mainly for superphosphate manufacture, but some is ground to a fine powder for use as a fertiliser. In addition to insoluble calcium

phosphate, the rock contains carbonate and often sulphate, fluorides, small percentages of iron, magnesium, silica, etc. The total phosphoric acid varies from about 27 to 40 per cent. The sale of this fertiliser must be accompanied by a statement giving the total amount of phosphoric acid, and the proportion of the material passing through the prescribed sieve.

Determination of Fineness and Phosphoric Acid. Follow the instructions as described for basic slag, but take a 50 ml. aliquot of the filtrate for the phosphate determination.

POTASSIC FERTILISERS

The potassic fertilisers used in this country are obtained mainly from the Stassfurt and Alsace deposits. During the war (1939-45) other countries, chiefly the U.S.A. and Palestine, supplied our requirements. There were five commonly used in this country prior to 1939 containing guaranteed amounts of potassium oxide (K_2O), which is the conventional unit for stating the amount of potash in fertilisers.

In kainit (14 per cent. K_2O) the potassium is present as chloride; sodium chloride is present in quantity, some magnesium sulphate, and traces of magnesium chloride and calcium sulphate are also present.

Potash salts are of two grades (20 per cent. K_2O and 30 per cent. K_2O) and may be regarded as refined grades of kainit, being composed mainly of potassium and sodium chlorides.

Muriate or chloride of potash[1] (50 to 52 per cent. K_2O) contains rather more than 80 per cent. of potassium chloride, and nearly 20 per cent. of sodium chloride.

Sulphate of potash (48 to 50 per cent. K_2O) contains about 90 per cent. of potassium sulphate with only traces of the salts usually present in the other potassic fertilisers.

Qualitative Examination. Note the appearance of the various potassic fertilisers and examine qualitatively for sodium, calcium, magnesium, chloride and sulphate.

[1] The muriate of potash obtained during the war (1939-45) contained 60 per cent. of potash equivalent to 95 per cent. of potassium chloride.

Determination of Potash. (*a*) *In absence of sulphates, e.g.* in muriate of potash. Weigh 4 gm. of the sample, dissolve in water and make up to 500 ml. Filter and determine the potash in 50 ml. of the filtrate with perchloric acid (p. 34). Use about 7 ml. of 20 per cent. perchloric acid for the first evaporation.

(*b*) *When sulphates are present.* Weigh into a beaker a portion of the sample equivalent in potash content to 1·5 to 2 gm. of potash (K_2O), *e.g.* 10 gm. of kainit or 4 gm. of sulphate of potash. Add about 280 ml. of water, 20 ml. of concentrated hydrochloric acid and boil. Add a solution of barium chloride drop by drop until the sulphates are completely precipitated, cool, make up to 500 ml. and filter. Evaporate 50 ml. of the filtrate to dryness. Cover the residue with concentrated hydrochloric acid and again evaporate to dryness. Extract the residue with hot water, filter and determine the potash (K_2O) in the filtrate with perchloric acid as above.

AGRICULTURAL SALT

Although neither sodium nor chlorine are usually regarded as essential elements for plant growth, the value of agricultural salt for sugar beet and mangolds is well established.

Determination of Sodium Chloride. (NaCl.) Weigh 5 gm. of the sample, dissolve in distilled water, transfer to a litre flask and make up to the mark. Pipette 25 ml. into a porcelain dish, add a few drops of potassium chromate as indicator and titrate with 0·1N. silver nitrate (see p. 32). Calculate the percentage of sodium chloride (NaCl) in the sample.

MIXED OR GENERAL MANURES

Potassium Nitrate. The use of this material is confined almost entirely to market gardeners. It is sold in several grades, one of which contains 12 per cent. of nitrogen and 44 per cent. of potash, and another 14½ per cent. of nitrogen and 15 per cent. of potash. The latter is a mixture of sodium and potassium nitrates.

The potash may be determined as above, and the nitrate nitrogen as described on page 46.

Bone Manures. These contain phosphate of organic origin,

and in addition small amounts of organic nitrogen. They include the following.

Bone Meal. In its preparation the fat is removed, and the bones ground to a rather coarse meal. It usually contains about 4 per cent. of nitrogen and 21 per cent. of phosphoric acid insoluble in water.

Steamed Bone Flour. In the production of this fertiliser most of the nitrogenous constituents are removed in addition to the fat, the residue being ground to a fine powder. It contains only about 1 per cent. of nitrogen, and from 27 to 30 per cent. of phosphoric acid, and for practical purposes may be regarded as a purely phosphatic manure.

Dissolved Bones. This product is manufactured from ground bones which are treated with sulphuric acid, and it usually contains about 2·5 per cent. of nitrogen and about 16 per cent. of phosphoric acid, of which more than half is water-soluble.

Spent Bone Charcoal. Bone charcoal is obtained by burning bones out of contact with air, and the product is used in refining raw sugar. The resulting spent bone charcoal is a purely phosphatic fertiliser, obtainable in several degrees of fineness, containing from 34 to 40 per cent. of phosphoric acid in an insoluble form, and about 10 per cent. of carbon. If the carbon is burnt off, the residue is bone ash which contains about 40 per cent. of phosphoric acid.

Examination of Bones and Bone Compounds. *Composition of Bones.* Weigh two whole bones separately. Place one in a muffle furnace and ignite at a bright red heat for about an hour, cool and weigh the residue, which is bone ash. Dissolve some of the ash in nitric acid and show that phosphate is present by adding a solution of ammonium molybdate.

Immerse the second bone in hydrochloric acid (one part strong acid to one part water) and leave for a few days. This dissolves the mineral matter of the bone, leaving behind a soft flexible piece of ossein or bone collagen. Wash the latter free from acid, dry in the oven and weigh. Show that the ossein contains nitrogen by heating a little with soda lime, and that it is a protein by applying the xanthoproteic reaction (p. 76). Boil the remainder of the ossein with distilled water for some minutes and note that it dissolves. Allow the solution to cool when it will set to a jelly, owing to the formation of gelatin produced from the bone collagen by the action of the water.

Vitriolised Bones. Weigh out 30 gm. of bone meal into a porcelain basin, mix with 15 ml. of water and then add gradually with constant stirring 5 ml. of concentrated sulphuric acid. The residue forms a solid but slightly damp mass of " vitriolised " or " dissolved " bones. Place some of the residue in a test tube, shake up with water, filter and test the filtrate for phosphate by adding ammonium molybdate solution.

Determination of Nitrogen and Insoluble Phosphoric Acid. These can be conveniently determined in the same weighed portion of the sample. Weigh out 5 gm. into a Kjeldahl flask, and treat as on p. 28. When digestion is complete allow to cool, dilute with water and wash out into a 500 ml. flask. Filter and determine the percentage of phosphoric acid and of nitrogen in aliquots of the filtrate (pp. 49 and 29).

Determination of Soluble Phosphoric Acid in Dissolved Bones. This is determined as described under superphosphate (p. 49).

Guano. True guanos consist of the residues of the excreta of fish-eating birds, and contain varying amounts of organic nitrogen, phosphoric acid and potash. Two grades are usually sold, one containing 10 to 14 per cent. of nitrogen and 9 to 11 per cent. of phosphoric acid, and the other 5 to 8 per cent. of nitrogen and 14 to 18 per cent. of phosphoric acid, while both grades contain from 2 to 6 per cent. of potash. Fish " guano " or fish manure contains from 6 to 10 per cent. of nitrogen and 5 to 9 per cent. of phosphoric acid, while meat " guano " or meat and bone meal contains from 3 to 8 per cent. of nitrogen and 10 to 20 per cent. of phosphoric acid.

Determination of Nitrogen and Phosphoric Acid. These are determined in the same weighed portion as described under bone manures.

Determination of Potash. Weigh 10 gm. of the sample into a porcelain dish and ignite gently to char organic matter. Allow to cool, add 20 ml. of concentrated hydrochloric acid and boil for a few minutes, covering the dish with a clock glass. Wash out into a beaker with water, making the final volume about 200 ml., and boil. Transfer the solution to a 500 ml. graduated flask, cool and make up to the mark with water. Filter and pipette 50 ml. of the filtrate into a beaker; add a little strong ammonia to partially neutralise, raise to the boiling-point and add powdered barium hydroxide until slightly alkaline. Cool slightly, add an excess of powdered ammonium carbonate and finally, while boiling, add a little powdered

ammonium oxalate. Filter while hot, wash the precipitate with hot water, first by decantation and then on the filter until free from chlorides. Evaporate the filtrate to dryness (nitric acid may be added during the evaporation after free ammonia has been driven off), and heat the residue gently over a low flame until all ammonium salts are expelled. Cover the residue with concentrated hydrochloric acid, evaporate to dryness, extract with hot water and filter. Determine the potash in the filtrate by precipitation with 5 ml. of perchloric acid, as described on p. 34.

Farmyard Manure and Poultry Manure. Farmyard manure is variable in composition, containing on the average from 0·4 to 0·6 per cent. of nitrogen, 0·2 to 0·3 per cent. of phosphoric acid and 0·5 to 0·7 per cent. of potash. Poultry manure is considerably richer, having an average composition of 1·5 to 2 per cent. of nitrogen, 1 per cent. of phosphoric acid and 0·5 per cent. of potash. Poultry manure is also dried and ground, but some nitrogen is lost in the drying process; the average composition of the dried product is $3\frac{1}{2}$ to 4 per cent. of nitrogen, 2 to 3 per cent. of phosphoric acid and 1 to $1\frac{1}{2}$ per cent. of potash.

Qualitative Examination. Either farmyard manure or poultry manure may be used.

Nitrogen. The nitrogen is present in various compounds, among which are ammonium salts, amides and other more complex organic compounds. Heat a few gm. of the manure with dilute sodium hydroxide in a test tube. Note that ammonia is given off, this being obtained from the ammonium salts and amides. When all the ammonia has come off, dry the residue, mix intimately with soda lime and again heat. Note that more ammonia is obtained indicating the presence of complex nitrogenous compounds (*e.g.* proteins).

Potash and Phosphoric Acid. Ignite about 20 gm. of the manure in a porcelain dish to obtain the ash. Extract about half of the ash with nitric acid, filter and test the filtrate for phosphate. Dissolve the remainder in dilute hydrochloric acid, filter, evaporate the filtrate to dryness, ignite the residue and test for potassium.

Determination of Total Nitrogen. The manurial value of farmyard manure or poultry manure depends largely on its nitrogen content, which may be determined by the Kjeldahl method (p. 28).

Weigh out 10 gm. of the fresh sample or about 4 gm. of the dried poultry manure, and use 30 ml. of concentrated sulphuric acid, 10 gm. of potassium sulphate and a crystal of copper sulphate. If farmyard manure was taken, distil the entire contents, but if poultry manure was taken (fresh or dried), dilute first to 500 ml. and use 100 ml. for the distillation. In each case take 50 ml. of 0·1N. acid in the receiving flask.

N.B.—If required, the percentage of phosphoric acid in the dried poultry manure may be determined in 200 ml. of the above solution.

Sewage Sludges. There are three main types, primary, digested and activated. Primary sludge is raw sludge which has been dried in lagoons or under-drained drying beds freely exposed to the air. Often the raw sludge is subjected before drying to an anaerobic process of " digestion " by methane forming bacteria. In another process the sewage is aerated in the presence of sludge already aerated or " activated " and this method produces a sludge of higher nitrogen content.

The value of sewage sludges depends mainly on their nitrogen content, although some contain appreciable amounts of available phosphate. The best guide to the availability of the nitrogen present is the percentage of nitrogen in the organic matter and generally speaking it is only the nitrogen in excess of about 3 per cent. in the organic matter which becomes available to plants. Raw primary sludge contains about $4\frac{1}{2}$ per cent. of nitrogen, digested sludge about 6 per cent. and activated sludge about 7 per cent. of nitrogen in the organic matter.

Determination of Organic Matter. Weigh a fairly large sample (10-20 gm.) in a porcelain dish and dry in an oven at 100° C. until constant in weight. Heat the residue over a bunsen burner to a bright red heat for half an hour or place in a muffle furnace for about 15 minutes. Cool and weigh, and from the loss in weight of the dry residue calculate the percentage of organic matter.

Determination of Nitrogen. From a large sample prepare a sub-sample and grind finely. Weigh 2 to 5 gm. into a Kjeldahl flask and proceed with the determination as described under poultry manure. Calculate the percentage of nitrogen in the original material and also in the organic matter.

COMPOUND FERTILISERS

There are many proprietary compound fertilisers on the market which are mixtures of various fertilisers. These should always be purchased with a guaranteed content of nitrogen, phosphoric acid and potash.

In general the methods of analysis of these are similar to those already given, except that if inorganic and organic nitrogen compounds are present together, modified methods must be used.

(*a*) If nitrates are present, the total nitrogen (organic, ammoniacal and nitrate) is determined by the modified Kjeldahl method described on p. 100.

(*b*) If ammoniacal nitrogen is to be determined in the presence of organic matter, proceed as for the determination of nitrogen in sulphate of ammonia, (p. 34) except that magnesium oxide (5 gm.) is used to drive off the ammonia.

Concentrated Compound Fertilisers. These recently introduced fertilisers differ from the old type of compound manures in that they contain nitrogen and phosphoric acid combined as ammonium phosphate, so that a much higher concentration of nitrogen and phosphoric acid is present than is possible in a simple mixture of, say, sulphate of ammonia and superphosphate. In these concentrated fertilisers the nitrogen is present as ammonium phosphate and sulphate of ammonia, the phosphoric acid as ammonium phosphate, and in some cases a little mineral phosphate. In the complete fertilisers, potash is added in the form of high grade potash fertilisers.

These may be analysed by the methods already given : nitrogen as in sulphate of ammonia (p. 44), soluble phosphoric acid as in superphosphate (p. 49), total phosphoric acid as in basic slag (p. 51), and potash as in potash fertilisers (p. 53).

LIME, HYDRATED LIME AND CARBONATE OF LIME

Burnt lime or quicklime is sold with a guaranteed content of calcium oxide (CaO) ; in hydrated lime or slaked lime the content of calcium hydroxide and its equivalent of calcium oxide

should be stated. Ground chalk and ground limestone are sold with a guaranteed content of calcium carbonate, and its equivalent of calcium oxide. In the case of ground limestone, the percentage which will pass through the prescribed sieve should be stated.

Qualitative Examination of Lime and Carbonate of Lime. A good sample of agricultural lime should be reasonably pure calcium oxide, with little or no hydroxide or carbonate present; while ground limestone or chalk or carbonate of lime should consist mainly of calcium carbonate. Lime and carbonate of lime should not contain much magnesia or insoluble matter.

(1) Test a sample of agricultural lime for the presence of carbonate.

(2) Test samples of agricultural lime and carbonate of lime for the presence of magnesium. Dissolve in dilute hydrochloric acid, boil and filter. Add ammonia until slightly alkaline and reacidify with acetic acid. Boil, precipitate the calcium with ammonium oxalate, filter and test the filtrate (concentrate if necessary) for magnesium by adding ammonium chloride, ammonia and sodium phosphate solutions.

(3) Note the amount of insoluble residue in (2), which will give an indication of the amount of insoluble silicates present.

Determination of Calcium Oxide in Quicklime or in Slaked Lime. The principle of this method is that calcium oxide and calcium hydroxide react with cane sugar to form calcium saccharate, which is fairly soluble, while any calcium carbonate and silicate which may be present do not react and remain insoluble. Calcium saccharate reacts with mineral acids in the same way as an equivalent amount of calcium oxide.

Accurately weigh about 5 gm. of the powdered sample in a weighing bottle and transfer to a stoppered bottle of about 1 litre capacity. Moisten with 10 ml. of alcohol (neutral to phenolphthalein) to lessen the possibility of caking, and add 490 ml. of a 10 per cent. solution of cane sugar (made neutral to phenolphthalein). Stopper immediately and agitate in a shaking machine for four hours. Filter through a dry filter paper into a dry vessel and titrate 50 ml. of the filtrate with 0·5N. hydrochloric acid, using phenolphthalein as

indicator. From the number of ml. of 0·5N. acid used, calculate the percentage of calcium oxide (CaO) in the sample.

1 ml. of 0·5N. acid = 0·014 gm. CaO.

Determination of Calcium Carbonate in Carbonate of Lime. Accurately weigh about 2·5 gm. of the sample into a beaker and add sufficient dilute hydrochloric acid to dissolve all of the carbonate present. Transfer to a 500 ml. flask, make up to the mark with water, shake well and filter through a dry filter paper. Determine the amount of calcium present (p. 17) in 25 ml. of the filtrate, and express the result as calcium carbonate ($CaCO_3$), and its equivalent of calcium oxide (CaO).

1 ml. of 0·1N. $KMnO_4$ = 0·0028 gm. CaO or 0·005 gm. $CaCO_3$.

Determination of Fineness in Ground Limestone. The same sieve is used as for basic slag, and the determination is similar to that given on p. 50.

CHAPTER III

PLANT AND ANIMAL BIO-CHEMISTRY

PLANTS and animals contain a large number of organic substances ; in the case of plants these are in the main elaborated from simple inorganic substances such as water, carbon dioxide and nitrates. In the case of animals, the starting-point for the building up of their typical products is the already highly complicated food derived from the plant world, directly, or via other animals.

The organic substances, whether plant or animal, may undergo various chemical changes, brought about mainly by the action of enzymes, *e.g.* in germination and ripening in plants, and in digestion and metabolism in animals. Many of the constituents of both plants and animals, such as proteins, carbohydrates, and enzymes, are colloidal in nature, a state intimately associated with vital processes. In this section the more important of the plant and animal products are included, and in particular those which are of importance in feeding stuffs.

CARBOHYDRATES

Carbohydrates are synthesised in plants from carbon dioxide and water. They constitute a large and very complex group of compounds, and may be roughly classified into sugars and non-sugars. In digestion, complex carbohydrates are changed into simple sugars, chiefly dextrose, which may be utilised to supply the animal organism with energy and to build up fats. Apart from dextrose, the only other important carbohydrate found in animals is glycogen, which is stored in the muscles and liver.

Sugars. There are two main groups, namely, monosaccharides with five or six carbon atoms (pentoses and hexoses), and disaccharides containing twelve carbon atoms, which on

hydrolysis give hexose sugars. The more important of the hexose sugars are dextrose, laevulose and galactose, while the chief disaccharides are sucrose, maltose and lactose.

Qualitative Tests for Dextrose or Glucose and Laevulose or Fructose ($C_6H_{12}O_6$). (*a*) To a small quantity of dextrose solution, add an excess of Fehling's solution (an alkaline solution of cupric tartrate), warm, and note the reddish precipitate of cuprous oxide indicating that reduction has taken place. Repeat the test with laevulose.

(*b*) To a weak solution of silver nitrate in a clean test tube, add dilute ammonia solution, drop by drop, until the precipitate that forms just redissolves. Add a small quantity of glucose solution or fructose solution and heat the test tube in a water bath. Note that a silver mirror is formed on the side of the tube, or finely divided silver is deposited in the solution.

(*c*) Fill two polarimeter tubes, one with dextrose solution and the other with laevulose solution. Place the tubes in the polarimeter, and note that the solution of dextrose is dextro-rotatory, and that of laevulose is laevo-rotatory.

Qualitative Tests for Sucrose or Cane Sugar and Maltose ($C_{12}H_{22}O_{11}$). Repeat the above tests with sucrose and maltose. Note that maltose reduces Fehling's solution and ammoniacal silver nitrate, but sucrose does not, and that both sugars are dextro-rotatory.

To about 5 ml. of solutions of maltose and sucrose (up to 1 per cent. concentration) add 3 or 4 drops of a 5 per cent. aqueous solution of methylamine hydrochloride. Boil for half a minute and at once add 3 to 5 drops of a 20 per cent. solution of sodium hydroxide. Note that no change occurs with sucrose and that a yellow colour quickly changing to carmine is obtained with maltose. This test, which is due to Fearon,[1] is only given by maltose and by lactose.

Hydrolysis of Sucrose. Sucrose is easily hydrolysed by dilute acids or by the enzyme sucrase or invertase, giving the reducing sugars dextrose and laevulose.

To a solution of cane sugar, add a few ml. of dilute hydrochloric acid, and boil for a few minutes. Neutralise the excess of acid with sodium hydroxide, and test with Fehling's solution and ammoniacal silver nitrate. Note the reduction in each case.

Distribution of Sugars in Plants. Sugars are very widely

[1] *Analyst* (1942), 67, p. 130.

distributed in plants, dextrose and laevulose chiefly in ripe fruits, while cane sugar occurs in much larger amounts in some plants, *e.g.* sugar cane and sugar beet.

Grate a sugar beet or mangold root and add warm water to the pulp. Stir for a few minutes, filter, and boil the filtrate with hydrochloric acid. Neutralise with sodium hydroxide, and test for reducing sugars with Fehling's solution.

Non-sugars or Polysaccharides. These are substances of high molecular weight, mostly amorphous and insoluble in water. Many carbohydrates are included in this group, and on complete hydrolysis hexoses or pentoses or both are obtained.

Starch $(C_6H_{10}O_5)_n$. Starch is produced in the leaves of all green plants, and is stored in seeds, roots and tubers as a reserve food material. The cereal grains and potato tubers serve as the main commercial sources of this substance. Starch exists in plants in the form of granules which vary in size and shape according to their source.

Preparation of Starch. Grate a few potatoes to a pulp, tie the latter in a cloth, dip in water and squeeze into a large beaker of water. The starch granules pass through the cloth and render the water milky. Allow the starch to settle and wash twice by decantation. Place on a porous tile and dry in the steam oven.

Qualitative Examination of Starch. (*a*) Shake up a little starch with cold water and note that it is insoluble. Warm the mixture and note that a viscous opalescent solution (a colloidal solution) is obtained.

(*b*) Dilute the starch solution obtained in (*a*) with a large volume of water, allow to cool, add a drop of iodine solution and note the deep blue colour produced. Boil the solution and note that the colour disappears and reappears again on cooling.

Dialysis of Starch. Colloidal substances are relatively indiffusible, while crystalloids diffuse readily through parchment papers or films of collodion. This property affords a means of separating colloids from crystalloids, the process being known as dialysis.

Prepare a 2 per cent. starch paste by rubbing up 2 gm. of starch with a few ml. of water in a mortar, pour into 100 ml. of boiling

water and continue to boil for about a minute. Mix the starch paste with about one-tenth of its volume of saturated ammonium sulphate solution. Pour the mixture into a diffusion shell, and place the latter in a beaker of water. Test the " dialysate " (the liquid in the beaker) at the end of half an hour for starch with iodine, and for sulphate by means of barium chloride. Note that the starch test is negative, while the sulphate test is positive. Test the dialysate again for starch after one or two days.

Hydrolysis of Starch. Starch is hydrolysed by dilute acids, ultimately forming glucose. In plants, especially during germination, the enzyme diastase hydrolyses starch to maltose ; in animals the ptyalin in saliva, and amylopsin in the pancreatic juice hydrolyse starch to maltose.

(*a*) *By Means of Acids.* Boil a little starch paste with some dilute hydrochloric acid for about ten minutes. Cool, neutralise the excess acid with sodium hydroxide solution, add Fehling's solution and warm. Note the reduction of the Fehling's solution.

(*b*) *By Means of Ptyalin* (*in Saliva*). Warm some distilled water in a beaker to about 40° C., and with a little of it thoroughly rinse out the mouth. Take about 20 ml. of the warm water into the mouth and move it about with the tongue for at least a minute. Collect this in a clean beaker and repeat the process twice more. Thoroughly mix the diluted saliva thus obtained and filter.

Prepare a 1 per cent. starch paste and a weak solution of iodine (about 0·02N.). Label two test tubes *A* and *B* ; to *A* add 5 ml. of 1 per cent. starch paste and 5 ml. of diluted saliva, and to *B* add 5 ml. of the starch solution and 5 ml. of the diluted saliva which has been previously boiled. Place in a water bath or large beaker of water maintained at about 40° C. Place a series of drops of iodine solution on a white porcelain tile, and from time to time transfer by means of a glass rod a drop of the digesting mixture to a drop of iodine. In the case of *A* note that the blue colour produced at first later becomes blue-violet, red brown and finally light brown. When a drop of the mixture gives no further colour with iodine, test about 5 ml. of the liquid for maltose by the methylamine test (p. 62).

(*c*) *By Means of Diastase* (*in Malt*). The enzyme diastase is present in seeds and increases in amount during germination. In the production of malt, barley is allowed to germinate for a

period of about ten days, and during this time the diastase increases very largely in amount.

Mix some malt in a beaker with hot water (about 70° C.) to make a thick porridge. Allow to stand for about an hour, and filter through a wad of glass wool. Test the filtrate for maltose by the methylamine test.

Test some ungerminated barley for starch and for maltose. Grind the grain to a meal, mix with hot water and filter through a wad of glass wool. Test separate portions of the filtrate with iodine and by the methylamine test.

Glycogen $(C_6H_{10}O_5)_n$. Glycogen is a white amorphous solid, very similar to starch, and is often called " animal starch ". It is an important reserve product in animals, being stored in the muscles and liver, but it has a very restricted distribution in plants, occurring chiefly in certain fungi.

Preparation. Mince some fresh liver, transfer to a beaker and stir with a 15 per cent. potassium hydroxide solution. Boil for about an hour, filter and add to the filtrate an equal volume of 96 per cent. alcohol. Filter off the precipitated glycogen and wash with a mixture of one part of 15 per cent. potassium hydroxide with two parts of 96 per cent. alcohol.

Qualitative Examination of Glycogen. (*a*) Stir up some glycogen with water in a beaker and warm. Note that it forms an opalescent solution. Reserve the solution for the following tests.

(*b*) To a little of the cooled solution add iodine solution drop by drop. Note the red colour obtained.

(*c*) To about 10 ml. of the solution add 20 ml. of alcohol and shake vigorously. Note that the glycogen is precipitated.

(*d*) Boil about 5 ml. of the solution with Fehling's solution. If pure no reduction is obtained.

Hydrolysis of Glycogen. Glycogen is hydrolysed by mineral acids to glucose, while diastatic enzymes hydrolyse it to maltose.

Boil about 20 ml. of a solution of glycogen with dilute hydrochloric acid for half an hour. Cool, neutralise with sodium hydroxide solution, add Fehling's solution and warm. Note the reduction indicating the presence of a reducing sugar.

Cellulose $(C_6H_{10}O_5)_n$. Cellulose is the main constituent of the framework of cells of plants ; in young plants pure cellulose

is present, but with increasing age encrusting materials are added. This process, called lignification, consists in the conversion of cellulose into lignocellulose, and is accompanied by a gradual disappearance of the protoplasm. The " fibre " of feeding stuffs consists of cellulose, which is the least digestible carbohydrate present. Cellulose is hydrolysed to glucose by concentrated sulphuric acid and by the enzyme cytase found in seeds. Examples of pure cellulose are filter paper and cotton wool.

Preparation of Cellulose. Place about 2 gm. of finely chopped straw in a beaker, add 200 ml. of water and 2 ml. of concentrated sulphuric acid. Boil for twenty minutes, filter through linen, using a filter pump, and wash the residue with hot water until free from acid. Replace the residue in the beaker, add 200 ml. of water containing 2·5 gm. of sodium hydroxide and again boil for twenty minutes. Filter through a cloth and well wash the residue with hot water. The residue is fairly pure cellulose, but is generally dirty grey in colour.

Qualitative Examination of Cellulose. (*a*) Tear up some thin strips of filter paper, place in a test tube and half fill with Schweitzer's reagent (a solution of copper hydroxide in ammonia). Cork the test tube and shake well until the paper dissolves. Pour the blue solution into a large bulk of water, and note that the cellulose is precipitated in a finely divided state.

(*b*) Repeat this experiment, using a solution of zinc chloride in acid (zinc chloride dissolved in twice its weight of hydrochloric acid). Note that the paper dissolves.

(*c*) To a few ml. of strong sulphuric acid in a basin slowly add filter paper, and note that it gradually dissolves. Pour the acid into about 100 ml. of water and boil for a few minutes to hydrolyse the cellulose sulphates which are converted into dextrose. Cool, neutralise the solution with a strong solution of sodium hydroxide, and test for the presence of a reducing sugar with Fehling's solution.

Lignin. Lignin is a very complex substance which contains a much higher percentage of carbon than cellulose. Lignin is easily detected in lignified tissues by certain colour reactions.

(*a*) Dissolve a little aniline in excess of dilute hydrochloric acid and soak some wood shavings, straw, or a match stick in the solution.

Note the bright yellow colour developed, indicating the presence of lignin.

(*b*) Dip wood shavings or straw in an alcoholic solution of phloroglucinol ; then dip in concentrated hydrochloric acid. Note that a crimson coloration is obtained.

Pectic Substances. Pectic substances or pectins are responsible for the jellying properties of fruit juices, and are of great importance in jam and jelly making. They are found in fruits, *e.g.* apples, currants and gooseberries and in fleshy roots, *e.g.* turnips, carrots, and beet. Many different names have been used for pectin and its related compounds, but they are now divided into three groups, protopectins, pectins and pectci acids. All forms when completely hydrolysed with mineral acids yield galactose and arabinose.

In unripe fruits or roots, protopectin, an insoluble substance, is present in association with cellulose as pectocellulose. This is the precursor of pectin, and as fruits ripen, protopectin is converted to pectin due to the action of an enzyme protopectinase.

Pectin ($C_{39}H_{58}O_{33}$) is soluble in water and occurs in the juices of ripe fruits and roots, and is the substance responsible for the jellying of such juices. It is methylated pectic acid, and can be hydrolysed by acid or alkali or by the enzyme pectase with the formation of pectic acid and methyl alcohol.

Pectic acid ($C_{35}H_{50}O_{33}$) occurs in over-ripe and decaying fruits, and is the fundamental compound of the pectic substances. It is insoluble in water and is incapable of forming a jelly with sugar and acid. With calcium salts, however, it forms calcium pectate and may form a gel.

Preparation of Pectin. Cut an apple (not quite ripe) into small pieces, place in a beaker, add a little water and boil. Filter through muslin, and precipitate the pectin by adding at least twice its volume of alcohol. Allow the gelatinous mass to stand for a short time, filter it off and wash with alcohol.

Test fruit juices for pectin content in the following way. To the fruit juice in a cylinder, add from three to four times its volume of alcohol and mix by inverting slowly. If a precipitate is thrown down in a solid mass sufficient pectin is present to make a jelly.

Qualitative Examination of Pectin. For the following tests a sample of dry powdered commercial pectin should be used.

(*a*) Shake a little pectin with warm water and note that it dissolves to give a viscous solution. Allow to cool and note that it does not form a jelly.

(*b*) To a solution of pectin, add a little sodium hydroxide solution and leave to stand for ten minutes. This hydrolyses the pectin, forming sodium pectate and methyl alcohol. Divide the solution into two parts. Acidify one part with concentrated hydrochloric acid, and note the gelatinous precipitate of pectic acid. To the other part add calcium chloride solution, and note the precipitate of calcium pectate obtained.

(*c*) Warm some pectin solution with strong hydrochloric acid to hydrolyse pectin to galactose and arabinose (a pentose sugar). Add a little phloroglucinol, and note the red colour characteristic of pentoses.

(*d*) Prepare a 1 per cent. solution of pectin in water, place about 40 ml. in a beaker, add 10 gm. of cane sugar and 0·2 gm. of tartaric acid. Stir and boil for a few minutes. Note that on cooling the resulting mixture sets to a gel (a jelly-like substance).

This test illustrates the physical change which occurs in the making of jams and jellies, in which precipitation of pectin as a gel occurs in the presence of the sugar added and the acid of the fruit.

ASCORBIC ACID OR VITAMIN C

Ascorbic acid is present in many plant tissues and is particularly abundant in many fruit juices, *e.g.* oranges and lemons. It is a derivative of gulose (a stereo-isomer of glucose), having the formula $C_6H_8O_6$. It is a powerful reducing agent and reduces iodine, silver nitrate, potassium permanganate and Fehling's solution. The usual quantitative determination depends upon the reduction of the dyestuff 2 : 6—dichlorophenol-indophenol. The following method for the detection of ascorbic acid in plant tissues depends upon the reduction of potassium ferricyanide and conversion of the resulting ferrocyanide into Prussian blue.

Prepare the following reagents (*a*) 8 per cent. acetic acid solution ; (*b*) 0·4 per cent. potassium ferricyanide solution, free from ferrocyanide ; (*c*) 0·25 gm. of anhydrous ferric sulphate dissolved by boiling with 20 ml. of water and 4·5 ml. of 85 per cent. phosphoric acid ; then adding a few drops of 1 per cent. potassium permanganate solution to give a faint pink colour, boiling again for a few minutes, cooling and diluting to 25 ml. No trace of blue colour should appear when equal volumes of the three reagents are mixed.

Cut up about 2 gm. of plant tissue (cabbage leaf, potato, apple, etc.) and place in a small porcelain mortar. Add 6 ml. of boiling 8 per cent. acetic acid and macerate with a pestle for a few minutes. Place one drop of the extract on a double filter-paper and add to the spot on the lower paper one drop of solution (*b*) followed by one drop of solution (*c*). In the presence of 0·003 mgm. of ascorbic acid in one drop (0·05 ml.) of the solution, a blue colour appears within half a minute ; with more than 0·005 mgm. the colour shows immediately, while if no colour appears within one minute absence of ascorbic acid in the extract is indicated.

N.B.—Fruit juices may be tested by this method using a suitable dilution of juice with cold 8 per cent. acetic acid.

FATS AND OILS

Fats and oils are widely distributed in plants and animals. The natural fats and oils (excluding the essential oils) are glycerides of fatty acids, generally of high molecular weight. The chief saturated acids present are palmitic acid ($C_{15}H_{31}COOH$) and stearic acid ($C_{17}H_{35}COOH$), while in some oils, acids of varying degrees of unsaturation are present, such as oleic acid ($C_{17}H_{33}COOH$), linoleic acid ($C_{17}H_{31}COOH$), and linolenic acid ($C_{17}H_{29}COOH$).

Qualitative Examination of Fats and Oils. The following tests should be carried out with olive oil, linseed oil and tallow.

(*a*) *Solubility.* Test the solubility by shaking with water, alcohol and ether respectively. Pour a few drops of the ethereal solution on to filter paper. The ether soon evaporates, leaving an oily stain.

(*b*) *Unsaturation.* Dissolve in ether, add bromine water, and note the degree of absorption of the bromine in each case. The combination of halogens with unsaturated glycerides is discussed on p. 156,

and a method is given for the quantitative determination of the "iodine value" of fats or oils. If time permits the student is advised to determine the "iodine values" of olive oil and linseed oil.

(*c*) *Emulsification.* An emulsion may be defined as a suspension of one liquid in another liquid in which it is insoluble. Use olive oil for the tests given below.

(1) Shake up some pure olive oil (free from oleic acid) with water. Note that the oil is broken up into small globules, but these soon coalesce again, and the oil forms a separate layer on the surface of the water.

(2) Repeat the tests but add a little soap solution before shaking. Note that the emulsion remains permanent because the oil globules become surrounded with a thin film of soap, which prevents them from coalescing.

(3) To about 10 ml. of pure olive oil in a test tube add 1 ml. of oleic acid, one drop of phenolphthalein solution and 0·1N. sodium hydroxide solution until the mixture is slightly alkaline. Shake vigorously, and note that a stable emulsion is formed, for a soap is produced by the combination of the oleic acid with the alkali and emulsifies the fat.

The emulsification of fat is of great importance in the digestion of fats in the duodenum. The fats of the food are emulsified due to the combined action of the alkaline bile, intestinal juice and pancreatic juice. The fat in the emulsified state has a much increased surface, and the consequent hydrolysis to glycerol and fatty acids by the steapsin of the pancreatic juice is greatly speeded up.

Hydrolysis of Fats. Fats may be hydrolysed to fatty acids and glycerol by (*a*) superheated steam, (*b*) acids, (*c*) alkalies and (*d*) enzymes.

By Alkalies. When fats are hydrolysed by alkalies, *e.g.* sodium hydroxide, the salts (soaps) of the acids are formed and glycerol is liberated; the process is called saponification. If an alcoholic solution of alkali is used, the rate of saponification is increased.

Place about 5 ml. of coconut oil or tallow in a small flask, add about 50 ml. of 10 per cent. alcoholic solution of sodium hydroxide

and 50 ml. of water. Boil under a reflux condenser until the whole of the oil has disappeared. Transfer the contents to a beaker, cool, add a few drops of phenolphthalein, dilute with water, and then gradually add dilute hydrochloric acid until the pink colour of the indicator almost disappears. To the liquid add common salt until a white precipitate settles out and then filter through linen. The precipitate consists of soap and the filtrate contains glycerol.

Show that the soap forms a lather with distilled water. To a little of the solution add dilute hydrochloric acid. Note the precipitation of a white fatty substance which consists of fatty acids from the soap.

Take a small portion of the filtrate containing the glycerol and exactly neutralise. Evaporate as far as possible on a water bath, dissolve the residue in alcohol and filter. Again evaporate the filtrate to dryness and heat the residue with a small quantity of potassium hydrogen sulphate. Note the smell of acrolein which is produced from the glycerol present.

By Enzymes. In most oil-containing seeds, enzymes termed lipases are present, which can hydrolyse fats ; an extract of the seeds of the castor bean is used commercially for the hydrolysis of fats. Lipases are present in the animal body, *e.g.* the pancreatic juice contains the lipase, steapsin, which hydrolyses the fat of the food during digestion. The bile salts aid in the emulsification of the fat, and also speed up the action of the steapsin. The hydrolysing action of steapsin and the effect of the bile salts is shown in the following experiment.

Label two test tubes *A* and *B* ; to *A* add 2 ml. of 1 per cent. solution of pancreatin and 1 ml. of water, and to *B* add 2 ml. of 1 per cent. pancreatin and 1 ml. of 1 per cent. solution of bile salts. To each add 5 ml. of olive oil emulsion prepared as above, stopper and shake thoroughly. Place the tubes in a beaker of water maintained at 40° C. for one hour and shake a few times during the digestion. Transfer the contents of the tubes to separate beakers, and rinse out the tubes with two successive portions of alcohol. Add 5 drops of phenolphthalein to each beaker, and titrate with 0·1N. sodium hydroxide, the number of ml. used being a measure of the fatty acids produced in the digestion. Note that the presence of the bile salts materially aids the digestion of the fat.

WAXES

Waxes occur in many plants, generally as a thin deposit on leaves, stems and fruit, acting as a protective layer against undue evaporation of water. Waxes are also found in the animal kingdom, the more important being wool wax (wool fat or lanolin), beeswax, spermaceti and Chinese insect wax.

Waxes resemble fats in being esters of the higher fatty acids, but the alcohol present is not glycerol. It is usually a monohydric alcohol of high molecular weight, *e.g.* cetyl alcohol ($C_{16}H_{33}OH$), ceryl alcohol ($C_{26}H_{53}OH$), cholesterol or phytosterol ($C_{27}H_{45}OH$).

Qualitative Tests with Beeswax. (*a*) Shake up a little of the wax with the ordinary fat solvents such as ether, benzene and petroleum ether, and note that it dissolves in each case.

(*b*) Heat a little with potassium hydrogen sulphate ; note that no smell of acrolein is obtained (distinction from fats).

STEROLS

When crude fats and oils are hydrolised with caustic alkalis, there always remains a small amount (usually not more than 0·5 per cent.) of material which is not hydrolysed. This is termed the unsaponifiable residue, and consists mainly of a group of alcohols known as sterols and fat soluble vitamins, if these were present in the original fat.

The sterols are monohydric secondary alcohols, and fall into two main groups, the cholesterols and the phytosterols, which are characteristic of the animal and vegetable world respectively.

Cholesterol ($C_{27}H_{45}OH$) occurs in the bile, gall stones, and butter fat, and may conveniently be prepared by extracting gall stones with ether, concentrating, and allowing to crystallise.

The term phytosterol was at one time used to designate a definite chemical individual of the formula $C_{27}H_{45}OH$, but is now used more as a generic term to include a number of different substances having certain properties in common. One of the best known is ergosterol ($C_{28}H_{43}OH$), first isolated from ergot,

but also present in yeast and in human and animal skins. It is of great importance in animal metabolism, because irradiation of ergosterol with ultra-violet light converts it into several isomers, including calciferol or vitamin D, the antirachitic vitamin.

Reactions of Cholesterol. (1) Place a few crystals of cholesterol on a white porcelain tile and moisten with sulphuric acid (five parts strong acid to one part of water). Warm if necessary and note the reddish-lilac colour produced.

(2) Dissolve a little cholesterol in chloroform and gently agitate with sulphuric acid (eight parts strong acid to one part of water). Note that the chloroform layer turns red, while the sulphuric acid layer assumes a green fluorescence.

(3) Dissolve a little cholesterol in alcohol, mix with a few drops of 1 per cent. alcoholic solution of digitonin and note the white precipitate produced. This reaction is employed in the determination of cholesterol or phytosterol and also in precipitating these substances prior to isolating them in a pure form (p. 158).

LIPINS

The term lipin is applied to a group of glycerol esters which in their physical and chemical properties are closely allied to the fats. They occur in all living cells in plants and animals, and are intimately connected with cell metabolism. The phospho-lipins (or phosphatides) contain fatty acids, glycerol, phosphoric acid and a nitrogenous base. In plants, they are present chiefly in protein-rich seeds ; while in animal tissues, egg-yolk, brain and nerve tissue are particularly rich. The most important members of the group are lecithins, of which the lecithin of egg yolk is a typical example. These, on hydrolysis, give fatty acids, glycerol, phosphoric acid and choline ; the formula of a typical example of the group is given below.

$$
\begin{array}{l}
CH_2.O.COC_{17}H_{35}. \\
| \\
CH.O.COC_{17}H_{33}. \\
| \\
CH_2.O.\underset{\underset{O}{\|}}{P}(OH).O.CH_2CH_2N(CH_3)_3OH.
\end{array}
$$

Qualitative Reactions of Lecithin. (1) To an alcoholic solution of lecithin, add an alcoholic solution of cadmium chloride, and note that a white precipitate is formed.

(2) Heat a little lecithin with soda lime and test for ammonia.

(3) Fuse a mixture of potassium carbonate and potassium nitrate in a crucible, add a little lecithin and heat until all carbon is burnt away. Allow to cool, dissolve the residue in dilute nitric acid, and test for the presence of phosphate with ammonium molybdate solution.

(4) Boil a little lecithin with sodium hydroxide solution. Note the characteristic fishy smell of trimethylamine. Acidify the solution with sulphuric acid, and note the white precipitate of fatty acids.

(5) Mix some lecithin with a small amount of water and note that it swells up, forming slimy threads (known as myelin forms). Add more water and note that these gradually dissolve, forming a colloidal solution.

PROTEINS

The proteins are a group of compounds containing carbon, hydrogen, oxygen, nitrogen and sulphur, and in some, phosphorus or iron is present. These compounds are present in the fluids and tissues of all living organisms, and are the chief constituents of protoplasm. In plants, proteins may occur either in the solid state as in seeds, or in solution in the cell sap. Chemically, they are composed of amino acids condensed together so that the amino groups are combined with the carboxylic groups; when hydrolysed the free amino acids are obtained.

Preparation of Gluten from Wheat. Moisten about 50 gm. of wheat flour with a little distilled water and make it into a stiff dough. Place the dough on a cloth and knead it well under a stream of water which carries away the starch. After a few minutes remove the mass from the cloth, and knead directly with the hands in the stream of water until all starch is removed, as shown by the absence of any milky appearance in the water draining from the mass. The gluten obtained is greyish yellow in colour and elastic, but should not be sticky. It consists of two proteins, glutenin and gliadin.

Qualitative Tests for Proteins. *Precipitation.* Proteins are precipitated from their solutions by various means. Prepare a solution of egg albumen by separating egg white from the yolk, well beat up with a whisk and dilute with four times its volume of distilled water.

(*a*) *By Heat.* Heat a small quantity of egg albumen solution, to which one drop of acetic acid has been added. Note that the albumen is coagulated, and precipitates as a flocculent precipitate.

(*b*) *By Alcohol.* To a solution of egg albumen add a large excess of alcohol, and note that the protein is precipitated.

(*c*) *By Salts of Heavy Metals.* To separate portions of egg albumen solution add a few drops of mercuric chloride solution and lead acetate solution. Note the white precipitate produced in each case.

(*d*) *By "Alkaloidal Reagents."* (1) To a solution of egg albumen add a solution of picric acid. Note the yellow precipitate formed. (2) Add a solution of trichloracetic acid to a solution of egg albumen. Note the white precipitate produced.

(*e*) **Reversible Precipitation.** Certain salts such as sodium chloride, magnesium sulphate and ammonium sulphate have the property of throwing proteins out of solution; the process is termed "salting out". This is, however, a purely physical phenomenon, and differs from chemical precipitation in that the proteins are precipitated unchanged, retaining all their original properties and solubilities. Saturated ammonium sulphate precipitates all proteins except peptones.

Add ammonium sulphate to a few ml. of egg albumen solution until saturated. Note the white precipitate formed. Dilute with a large volume of water and note that the precipitate dissolves.

Tests for Elements Present. *Nitrogen.* (*a*) Heat a little egg albumen and wheat gluten in separate test tubes with soda lime. Note that ammonia is given off in each case.

(*b*) In a small hard glass test tube, place a small quantity of egg albumen or gluten with a small piece of metallic sodium. Heat until the temperature is raised to bright redness, remove the test tube from the flame, and while still hot drop the test tube into a few ml. of cold water in a porcelain dish. Break up the tube into small pieces, well mix the contents and filter. To a portion of the filtrate, add a few drops of ferrous sulphate solution, acidify with strong hydrochloric acid and finally add a few drops of ferric chloride solution. Note the greenish blue or blue coloration or the precipitate of Prussian blue which is produced.

In this test the sodium combines with the nitrogen and some carbon of the protein to form sodium cyanide, which with ferrous sulphate forms sodium ferro-cyanide. The addition of ferric chloride to the acid solution results in the precipitation of ferric ferrocyanide or Prussian blue.

$$6NaCN + FeSO_4 = Na_4Fe(CN)_6 + Na_2SO_4.$$
$$3Na_4FeC_6N_6 + 4FeCl_3 = Fe_4(FeC_6N_6)_3 + 12NaCl.$$

Sulphur. To a portion of the filtrate obtained above (in nitrogen test (*b*)) add a few drops of a weak solution of sodium nitroprusside. Note the violet coloration produced owing to the presence of sodium sulphide formed by the combination of the sodium with the sulphur of the protein.

Phosphorus. In a crucible place a little of a mixture of equal parts of potassium carbonate and potassium nitrate. Heat the mixture, and while fused drop in small quantities of casein or vitellin (proteins containing phosphorus). Rapid oxidation of the protein takes place, the phosphorus present being oxidised to potassium phosphate. Allow the fused mass to cool, extract with hot dilute nitric acid and filter. To the filtrate add ammonium molybdate solution, warming if necessary, and note the yellow precipitate produced, indicating the presence of phosphate.

Colour Reactions for Proteins. These reactions depend on the presence of some specific amino-acid or amino-acid grouping in the protein molecule, with the exception of the biuret reaction, which depends on the constitution of the protein molecule. Characteristic colours are produced with suitable reagents, and although some of the reactions are given by all proteins, other tests are responded to only by a certain number. The following tests should be carried out with egg albumen, gluten and casein.

(*a*) *Xanthoproteic Reaction.* Add a little concentrated nitric acid to a protein or its solution and warm. Note that the white precipitate first produced turns yellow and partly dissolves to give a yellow solution. Cool, add strong ammonia solution until alkaline, and note that the yellow colour is changed to orange.

The yellow colour in this reaction is due to the action of the nitric acid on some aromatic group in the protein molecule with the formation of a nitro-derivative. These aromatic substances are tyrosine, tryptophane and phenylalanine.

(*b*) *Millon's Reaction.* To the protein or its solution add a little Millon's reagent and warm. The white precipitate first produced turns red or a red colour is obtained.

This reaction is given by all substances containing a phenolic group, and the substance derived from protein which is responsible for the reaction is tyrosine. Proteins (*e.g.* gluten) which do not contain tyrosine do not give the reaction.

(*c*) *Biuret Reaction.* To the protein, or a solution of the protein, add strong sodium hydroxide solution, warm and add one drop of a weak solution of copper sulphate. Note the bluish-violet, violet or pink colour produced and compare with a control test using water in place of the protein solution.

This reaction is given by biuret ($NH_2CO.NH.CONH_2$), and by similarly constituted compounds containing two $-\overset{\overset{O}{\|}}{C}-\overset{\overset{H}{|}}{N}-$ groups attached to one another, to the same nitrogen atom, or to the same carbon atom. The last-named grouping—$CO.NH.\overset{\overset{|}{}}{C}H.CO.NH$—occurs with great frequency in proteins, which, consequently, give this colour reaction.

(*d*) *Adamkiewicz's or the Glyoxylic Reaction.* Dissolve the protein in glacial acetic acid containing a trace of glyoxylic acid ("reduced oxalic acid"). Pour concentrated sulphuric acid carefully down the side of the tube, and note the purple ring where the two liquids meet.

This reaction is due to the presence of tryptophane (indol-amino-propionic acid) in the protein molecule, and is not given by proteins which do not contain tryptophane.

Hydrolysis of Proteins. Proteins can be hydrolysed by boiling acids or alkalis and by the action of certain enzymes, *e.g.* pepsin, trypsin and erepsin, which are present in the digestive juices. Soluble substances simpler than proteins are successively produced, *e.g.* proteoses, peptones and peptides, and ultimately if hydrolysis is complete, amino acids are obtained.

By Pepsin and Hydrochloric Acid. Pepsin is the proteolytic enzyme present in the gastric juice—it is secreted in the zymogen form, pepsinogen, which is converted to pepsin by hydrochloric acid. Pepsin is only active in a decidedly acid medium,

the optimum *p*H (p.11) being about 1·4. For the following exercise use fibrin or finely divided coagulated egg-white.

Place an equal amount of the protein in each of four test tubes and add the following :

(1) 5 ml. of 1 per cent. pepsin solution and 5 ml. of 0·8 per cent. HCl.
(2) 5 ml. of 1 per cent. pepsin solution and 5 ml. of water.
(3) 5 ml. of water and 5 ml. of 0·8 per cent. HCl.
(4) 5 ml. of pepsin solution that has been previously boiled and 5 ml. of 0·8 per cent. HCl.

Place the tubes in a water bath or large beaker of water at 38° to 40° C. for at least half an hour.

Note that in (1) the protein swells up, becomes transparent and finally dissolves, in (2) the protein is unaltered, in (3) and (4) the protein swells up, but does not dissolve.

These exercises show that neither 0·4 per cent. HCl alone, nor pepsin alone, can digest protein, but that pepsin in the presence of 0·4 per cent. HCl has this property. In (4) the enzyme pepsin has been destroyed by boiling.

AMINO ACIDS

Amino acids are compounds in which a hydrogen atom of an alkyl group of an organic acid has been replaced by an amino group.

E.g.

Acetic acid. Amino-acetic acid (Glycine).

All of the amino-acids obtained in the hydrolysis of proteins have the amino group attached to the same carbon atom as that to which the carboxylic group is attached, *i.e.* they are α-amino-acids. The presence of the —NH_2 group in amino acids confers upon these substances basic properties in addition to the acid properties due to the COOH group. Perform the following tests with a sample of glycine.

(*a*) Show that it dissolves in water, giving a neutral solution.

(*b*) Heat some with soda-lime in a test tube and note that ammonia is given off.

(*c*) To a solution of about 0·5 gm. of glycine in about 40 ml. of distilled water, add an excess of freshly precipitated, well washed cupric hydroxide. Boil for five minutes, filter, and concentrate the filtrate in an evaporating dish on a water bath. Set the dish aside, and note that in time fine blue needle-shaped crystals of the copper salt are formed.

This test shows that amino-acids combine with bases to form salts. It can also be shown that they combine with acids such as hydrochloric, to form salts.

(*d*) To a solution of glycine add dilute hydrochloric acid and sodium nitrite solution. Note the effervescence due to evolution of nitrogen.

$$CH_2.NH_2.COOH + HNO_2 = CH_2OH.COOH + N_2 + H_2O.$$

(*e*) Weigh approximately 0·1 gm. of glycine and dissolve in about 20 ml. of water in a boiling tube. Add a few drops of phenolphthalein solution, and titrate with 0·1N. sodium hydroxide until a faint pink is obtained. Add about 1 ml. of formalin solution and titrate again until a faint pink is obtained.

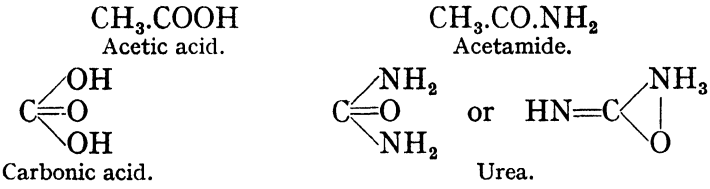
This exercise shows that amino acids which have been neutralised, react with aldehydes (in this case formaldehyde) to form compounds which are acid. The reaction is :

$$\begin{array}{l} CH_2.NH_2 \\ | \\ COOH \end{array} + HCHO = \begin{array}{l} CH_2.N=CH_2 \\ | \\ COOH \end{array} + H_2O.$$

The formaldehyde combines with the NH_2 group to form a methylene group which is neutral ; the whole molecule now behaves as an acid, owing to the unopposed influence of the terminal —COOH groups. This reaction is the basis of a method of determining the proteins in milk (p. 136).

AMIDES

Amides are widely distributed in plants, especially asparagine and glutamine. Urea, the most important form of waste nitrogen excreted by the animal organism, is also found in small amounts in a variety of plants, viz. spinach, potato, wheat and soya bean. Amides are characterised by containing the group —$CONH_2$, and may be considered as being formed from acids by the replacement of an —OH group by the —NH_2 group.



Separation of Urea from Urine. Evaporate about 30 ml. of urine to complete dryness on a water bath. Turn out the flame, and rub the residue with about 10 ml. of acetone until it is boiling. Allow the acetone to boil, stirring all the time, until about half of it has evaporated. Pour off the acetone into a dry watch glass, allow to cool, and note that crystals of urea separate out as silky needles.

Qualitative Test for Amides. The following tests should be performed with acetamide.

(*a*) Dissolve a small quantity in water, add sodium hydroxide solution and boil. Note that ammonia is given off.

$$CH_3CONH_2 + NaOH = CH_3.COONa + NH_3.$$

(*b*) Dissolve a small quantity in water, add dilute sulphuric acid and boil vigorously. Note that the steam smells of acetic acid, and is acid to litmus paper.

$$2CH_3CONH_2 + 2H_2O + H_2SO_4 = 2CH_3.COOH + (NH_4)_2SO_4.$$

(*c*) To a solution of acetamide, add a small quantity of sodium nitrite solution and a little dilute hydrochloric acid. Note the effervescence caused by the evolution of nitrogen gas.

$$CH_3.CONH_2 + HNO_2 = CH_3.COOH + H_2O + N_2.$$

Perform the following Tests with Urea. (*a*) Test the solubility in water, alcohol and ether.

(*b*) Perform tests (*a*) and (*c*) as for acetamide—note that ammonia is given off in (*a*) and that an effervescence of gas occurs in (*c*).

$$CO(NH_2)_2 + 2NaOH = Na_2CO_3 + 2NH_3.$$
$$CO(NH_2)_2 + 2HNO_2 = CO_2 + 2N_2 + 3H_2O.$$

(*c*) To a strong solution of urea add a few ml. of concentrated nitric acid, and note the white precipitate of urea nitrate.

(*d*) Place a little urea in a dry test tube and heat carefully over a flame, keeping the upper part of the tube cool. The urea melts and evolves ammonia, and a white sublimate which contains biuret condenses on the cooler parts of the tube. Dissolve in water, add a little sodium hydroxide, and a drop of weak copper sulphate

solution. Note the violet red coloration produced (biuret test, p. 77). Biuret is formed when urea is heated, according to the equation :

$$\underset{\text{Urea.}}{NH_2.CO.NH_2} + H.NH.CO.NH_2 = \underset{\text{Biuret.}}{NH_2.CO.NH.CONH_2} + NH_3.$$

Specific Urease Test for Urea. (*e*) The enzyme urease, which is widely distributed in plants, decomposes urea into ammonia and carbon dioxide. It occurs especially in the Leguminosae, being present in relatively large amounts in the soya bean.

To 5 ml. of a dilute solution of urea add 5 drops of phenol red. The colour obtained should be slightly pink. Add a drop or two of very dilute acetic acid by means of a glass rod until the reaction is very faintly acid to the indicator. Warm to about 45° C., add about a gm. of finely ground soya bean meal, shake and keep the solution warm. Note that the colour changes to a reddish purple, owing to the enzyme urease converting neutral urea to alkaline ammonium carbonate.

On the cyclic form of urea, urease is a dissociating enzyme, giving ammonia and cyanic acid, $HN=C=O$; the latter is then hydrolysed by the water present to give another molecule of ammonia and carbon dioxide, the ultimate product being ammonium carbonate.

$$HN=C\begin{cases}N.H_3\\ |\\ O\end{cases} \rightleftharpoons HN=C=O+NH_3.$$

$$HN=C=O+H_2O \rightleftharpoons NH_3+CO_2.$$

URIC ACID ($C_5H_4N_4O_3$)

Uric acid or salts of uric acid are normal constituents of urine, being obtained from the katabolism of proteins in the animal body. It is found particularly in the urine of birds, where it forms the chief nitrogenous excretory product, and is also present in human urine. It belongs to the class of compounds known as purines.

Qualitative Tests for Uric Acid. (*a*) Shake up a little with water and note that it is almost insoluble. Warm the solution and note that some, if not all, dissolves.

(*b*) To a small quantity of uric acid add dilute sodium hydroxide,

warm and note that the uric acid dissolves. Acidify with hydrochloric acid, cool and note that the uric acid crystallises out.

(*c*) To a small quantity of uric acid in a porcelain dish add a few drops of strong nitric acid. Heat on a water bath until all trace of nitric acid and water has been removed. Note the reddish deposit which remains, and treat this with a dilute solution of ammonia. Note that the residue turns reddish violet in colour. This test is known as the murexide test.

To Demonstrate the Presence of Uric Acid in Urine. (Use human urine). Treat 50 ml. of urine with two drops of ammonia, and then stir with powdered ammonium chloride until the solution is saturated. Allow the excess of ammonium chloride to settle, and pour off the supernatant liquid into another beaker. Note the gelatinous precipitate of ammonium urate. Filter off the precipitate and transfer it to an evaporating dish. Add three or four drops of concentrated nitric acid and evaporate on a water bath until a pink dry residue is obtained. Add a little dilute ammonia solution to the residue and note the purple colour produced (murexide test), thus indicating the presence of urates in the original urine.

GLUCOSIDES OR GLYCOSIDES

Glucosides are very widely distributed in plants. When hydrolysed by acids or enzymes they give various organic compounds, and a sugar which is usually glucose. Other sugars, however, are sometimes present, and because of this the general name glycoside has been suggested for the class of compounds. Among the more important glucosides are the cyanogenetic (cyanophoric) ones, so named because they yield hydrocyanic acid (prussic acid) on hydrolysis. The hydrolysis can be brought about by dilute acids or by the appropriate enzyme. When the specific enzyme occurs in the same tissue, bruising the material, or autolysing it with chloroform, is sufficient to effect the hydrolysis, and the hydrocyanic acid may be detected by appropriate tests.

Amygdalin ($C_{20}H_{27}NO_{11}$). This cyanogenetic glucoside occurs in the seeds of the bitter almond, and in kernels of the peach, plum, apricot and cherry laurel. It is hydrolysed by dilute acids or by

the enzyme " emulsin " (composed probably of three enzymes) into benzaldehyde, prussic acid and glucose.

$$C_{20}H_{27}NO_{11} + 2H_2O = C_6H_5CHO + HCN + 2C_6H_{12}O_6.$$

Hydrolysis of Amygdalin. (*a*) *By Emulsin.* Grind up a few almonds, soak in water, and allow to stand in a stoppered test tube in which freshly prepared congo red-silver nitrate papers are suspended. Keep in a warm place for about half an hour and note that the papers turn blue, indicating the presence of prussic acid.

(*b*) *By Dilute Acids.* Hydrolyse a very small quantity of amygdalin by boiling with dilute sulphuric acid in a test tube. Show that the vapours contain prussic acid by testing with congo red-silver nitrate papers or by means of a glass rod moistened with silver nitrate solution. The silver nitrate will become opalescent owing to the formation of a precipitate of silver cyanide.

Test the solution in the test tube with Fehling's solution (first neutralising with sodium hydroxide), and note that this is reduced by the benzaldehyde and glucose which have been produced.

Phaseolunatin ($C_{10}H_{17}O_6N$). This cyanogenetic glucoside occurs in the seeds of wild plants of phaseolus lunatus (Burmah bean), and in the flax (linseed) plant. A specific enzyme linase accompanies it in the flax plant, which hydrolyses the glucoside to acetone, prussic acid and glucose.

$$C_{10}H_{17}O_6N + H_2O = CH_3.CO.CH_3 + HCN + C_6H_{12}O_6.$$

Hydrolysis of Phaseolunatin by Linase. Place a few gm. of crushed linseed with warm water (about 38° C.) in a small flask, and insert a strip of congo red-silver nitrate paper with the cork. Leave in a warm place for a few hours and note if any prussic acid is produced.

A quantitative method for the determination of prussic acid in linseed cake is given on p.104.

THE ESSENTIAL OILS

Almost all plants contain fragrant volatile products which can be isolated by steam distillation. These are termed the essential oils in contradistinction to the fixed oils. They are found especially in flowers, *e.g.* lavender ; in fruits, *e.g.* citrus ; and in leaves, *e.g.* eucalyptus. The essential oils are usually

mixtures of compounds belonging to varied classes, *e.g.* hydrocarbons, esters, aldehydes, alcohols and phenols. Many of the constituents belong to the alicyclic group of compounds, which have as their base cyclo hexane (C_6H_{12}), which has a ring structure like benzene but is completely hydrogenated.

Preparation of Essential Oils. Place some heads of lavender or eucalyptus leaves in a distilling flask, half fill with water and fit with a condenser and receiver. Distil off about one-third of the water ; the distillate will contain a large proportion of the essential oil, which is volatile in steam.

Extract the distillate in a separating funnel with small quantities of ether, and separate the ethereal solution from the aqueous portion. Distil off the ether on a water bath, and note that the residue of essential oil possesses the characteristic odour of the plant taken.

PIGMENTS

The pigments in plants can be divided into two groups, depending on where and how they occur : (*a*) the plastid pigments, and (*b*) the cell-sap or soluble pigments. The former comprise two types of pigments, the chlorophylls, which give the green colour to plants, and the carotinoids or lipochromes, which are responsible for many of the yellow and orange hues in nature. They are insoluble in water, but soluble in fats and in fat solvents. The cell-sap pigments are soluble in water and include two groups, the anthoxanthins and the anthocyanins, which are not considered here.

Chlorophyll. Chlorophyll plays an essential part in the process of photosynthesis, by acting as the transformer of part of the light energy falling on the leaf, into the chemical energy necessary for the reduction of carbonic acid to carbohydrate. In the latter, or in products ultimately manufactured from it in the plant, the energy so obtained is stored up.

As ordinarily isolated, chlorophyll is a mixture of two substances, chlorophyll *a* ($C_{55}H_{72}N_4Mg$) and chlorophyll *b* ($C_{55}H_{70}O_6N_4Mg$).

Extraction of Chlorophyll. Plunge some green leaves into hot

water and then cut up in a beaker. Add warm alcohol and pour off the solution of chlorophyll obtained.

Properties. (*a*) Note that the solution shows a marked red fluorescence.

(*b*) Add a few drops of concentrated nitric acid to some of the chlorophyll solution. Note the disappearance of the colour of the chlorophyll due to oxidation.

(*c*) Examine some of the solution through a spectroscope and note the characteristic spectrum.

Carotinoids or **Lipochromes.** These occur in both plants and animals ; in the latter they are derived from, although they are not always identical with, the plant carotinoids in the food. They are all soluble in the " fat solvents ", and remain in the unsaponifiable residue. The two most important groups of carotinoids are the carotenes, and the xanthophylls. In animals they are responsible for the yellow colour of fats, hence the term lipochrome. The yellow pigment in the fat of the milk of the cow is carotene, while that of egg yolk and flesh of fowls is xanthophyll.

Carotene ($C_{40}H_{56}$). This was first isolated from the roots of the carrot, where it occurs in comparatively large amounts. Carotene exists in several isomers, and is important in the animal world as it is the precursor of vitamin A. Carotene can be converted into vitamin A ($C_{20}H_{30}O$) in the animal organism. The vitamin arises from a partial oxidation of the pigment, β-carotene being the isomer most effectively converted.

At present the valuation of dried grass is partly based upon its content of β-carotene. This may contain an isomer pseudo-α-carotene in equilibrium with it but having the same biological activity. On the other hand, the pigment neo-β-carotene present in fresh leaf material has little if any biological activity.

Extraction of Carotene. Melt some butter (free from added colouring matter) and pour a little of the molten fat into a test tube. Add about four times its volume of petroleum ether, shake to dissolve, and note the yellow colour of the solution which is due to carotene. Winter butter is generally poor, while summer butter is rich in carotene.

Xanthophylls ($C_{40}H_{56}O_2$). These substances are closely related to carotene and exist in several isomeric forms. They can be regarded as dihydroxy derivatives of carotene.

Extraction of Xanthophylls. Shake up a little egg yolk with acetone in a test tube and boil. Filter, and note the coloured filtrate which is due to the presence of xanthophylls.

Separation of Plastid Pigments. The principles involved in the separation of the pigments can be followed qualitatively by an adaptation of the quantitative methods evolved by Barton Mann.[1] It will be noted that in the main, the separation is effected by the preferential adsorption by bone-flour of individual pigments, from a mixture of them dissolved in an appropriate solvent.

All Plastid Pigments

(Soluble in a mixture of acetone, and ether)

↙ ↘

Chlorophylls, neo-β-carotene and Xanthophylls (Adsorbed from a solution in petroleum by bone flour)

β-carotene (Not adsorbed from a solution in petroleum by bone flour)

↓

Chlorophylls (Soluble in water after saponification) → **Xanthophylls** (Adsorbed from a solution in benzene by bone flour) → **Neo-β-Carotene** (Not adsorbed from a solution in benzene by bone flour)

Place about 3 gm. of finely ground dried leaves or dried grass in a Soxhlet thimble and extract with about 70 ml. of a mixture of 1 part acetone with 3 parts ether. This is best carried out by placing the thimble in a straight-through drip-type extraction tube fitted with a round bottomed flask and reflux condenser. By means of a water bath maintain for 30 minutes a temperature just sufficient to boil the acetone-ether mixture.

After 30 minutes distil off the solvent and immediately the distilling flask is dry, cool it under the tap and dissolve the contents in about 30 ml. of petroleum ether.

[1] *Analyst* (1944), 69, p. 34.

When dissolved, percolate the solution through a 3 in. column of prepared steamed bone flour[1] contained in a tube about ¾ in. diameter (a large straight calcium chloride tube is convenient) and which is fitted to a small filter flask attached to a water pump. The eluate contains *β-carotene.*

Elute the chlorophylls, neo-β-carotene and xanthophylls from the bone meal on which they are adsorbed, by passing through it about 100 ml. of ether. Return this eluate to the original flask and remove the ether by distillation. Add 5 ml. of a freshly prepared 30 per cent. solution of potassium hydroxide in methyl alcohol and heat under a reflux condenser for 10 minutes. Cool the flask, add about 50 ml. of ether, transfer its contents to a separating funnel containing about 70 ml. of water, shake gently and allow to stand. The water phase contains the *chlorophyllins* resulting from saponification of the chlorophylls ; the ether phase contains neo-β-carotene and xanthophylls.

Run off the aqueous bottom layer and wash the ethereal layer in the funnel with water until the washings give no colour with phenolphthalein. Transfer to a distillation flask, and distil off the ether, dissolve the residue in about 30 ml. of petroleum ether and percolate through a fresh column of bone flour to adsorb both pigments. Replace the filter flask by a clean dry one and elute the bone flour with benzene until the eluate runs through colourless ; this eluate contains the *neo-β-carotene.* The *xanthophyll,* which is adsorbed by the bone flour, can now be dissolved from it by extraction with acetone.

BLOOD

Clotting. Blood which escapes from a blood vessel usually clots quickly and a pale yellow serum gradually exudes from the clot. The phenomenon of clotting is very complex, but the factors concerned include thrombokinase, which in the presence of calcium ions produces the enzyme thrombin from its precursor prothrombin. The thrombin then acts on fibrinogen, a soluble protein of the blood, to produce fibrin, which being insoluble in plasma is precipitated and with the corpuscles forms the clot. The process of clotting or coagulation does not occur in

[1] Commercial steamed bone flour which has been extracted with acetone and ether to remove fat, sterols, etc.

the absence of calcium ions and consequently it can be prevented by precipitating the calcium salts of blood *e.g.* as calcium oxalate.

Prepare some oxalated blood by drawing the blood into 20 per cent. potassium oxalate (1 ml. to 100 ml. blood) with continuous shaking.

Examine a drop of oxalated blood under the microscope. Notice the red cells, which singly appear reddish-yellow, and the fewer and larger white cells. Add 2 drops of distilled water. The cells absorb water and swell and finally rupture (haemolysis), the coloured material (haemoglobin) being liberated and diffusing through the solution. This rupture of the cells is also known as the process of "laking".

Centrifuge 10 ml. of oxalated blood for fifteen minutes. Only partial separation of cells and "oxalated plasma" will have been effected in this short period. Pour off the clear upper layer, add 2 drops of a saturated solution of calcium chloride to it and allow it to stand. Note that a colourless clot forms and that on standing the clot shrinks, and serum exudes. Hasten the process by stirring up the clot with a glass rod. A white mass of fibrin adheres to the rod. Transfer this mass to a test tube and wash it with water. Then test separate minute portions of it as on p. 76, and so demonstrate its protein nature.

The above experiment shows the necessity of calcium ions for clotting, and that the process is due to constituents present in the plasma.

Blood Pigments. The pigment of the red blood corpuscles named haemoglobin plays an essential part in the respiration of animals. It is formed by the union of a protein called globin with a pigmented non-protein substance containing iron and called haematin. With the oxygen of the air and consequently in the blood it forms the compound oxyhaemoglobin and with carbon monoxide the much more stable compound carboxyhaemoglobin.

Haemoglobin. Take some blood or haemoglobin solution in a test tube and shake well with air to form oxyhaemoglobin. Add a drop or two of ammonium sulphide, warm gently and notice a rapid change in colour from scarlet red to a dark purplish-red. Shake up

with air. The original colour gradually reappears. The oxyhaemoglobin (scarlet-red) was reduced by the sulphide to "reduced" haemoglobin (purplish-red), which latter absorbed oxygen from the air again to form oxyhaemoglobin.

Carboxyhaemoglobin. Pass a slow stream of coal-gas (which contains carbon monoxide) through 3 ml. of blood or haemoglobin solution in a test tube, carrying out the operation in a fume-chamber. Notice the change of colour to cherry-red, due to formation of carboxyhaemoglobin. Repeat the test with ammonium sulphide given in the previous exercise and note that carboxyhaemoglobin cannot be reduced and then oxygenated when shaken up with air.

Blood Constituents. Blood contains mineral matter (chiefly sodium chloride), proteins, lipoids and a little glucose. For the following tests prepare some defibrinated blood by collecting blood in a small bottle containing a glass bead and shaking immediately. The fibrin adheres to the bead and can be separated from the remainder of the blood which does not clot.

(*a*) **Mineral Constituents of Plasma.** Centrifuge 10 ml. of defibrinated blood, decant the serum and remove proteins by boiling and adding 2 drops of dilute acetic acid. Filter and test portions of the protein-free filtrate for chloride, phosphate and calcium.

(*b*) **Glucose.** To 4 ml. of the filtrate prepared in (*a*) add 4 drops of Benedict's solution and boil for 1 minute. Note the red, yellow or green precipitate produced, depending on the concentration of glucose present.

(*c*) **Iron.** Take 5 ml. of defibrinated blood and evaporate to dryness in a porcelain dish. Ignite to remove most of the carbon, dissolve the residue in dilute hydrochloric acid, filter and test the filtrate for iron.

Repeat this test with plasma or serum and with red corpuscles after centrifuging. Note that the test for iron is only given by the red corpuscles.

EGGS

Proximate Analysis of Eggs. Weigh an egg, and then separate the contents into shell, yolk and white. Transfer each to separate weighed dishes and weigh, thus obtaining the weight of shell, yolk and white respectively. Dry each dish in an oven at 100° C. until

constant in weight and weigh again. Calculate the percentage of dry matter in each case. Grind up the contents of each dish and examine as follows :

Shell. *Organic Matter.* Weigh a small evaporating dish, add about 2 gm. of powdered shell (dry matter) and weigh again. Ignite over a bunsen burner, until the small amount of organic matter has burned away, cool and weigh. The loss in weight represents the organic matter of the shell ; express this as a percentage (*a*) of the dry shell, (*b*) of the original shell.

Calcium Carbonate. Cover the dish from the previous experiment with a clock glass, and add sufficient dilute hydrochloric acid to dissolve the ash. Transfer the solution to a 500 ml. flask, rinse out the dish and the clock glass with distilled water, and make up to the mark. Shake the contents thoroughly and pipette 25 ml. of the solution into a beaker. Make slightly alkaline with ammonia, and add sufficient acetic acid to dissolve the precipitate formed. Dilute to about 100 ml. and complete the determination as on p. 17. Express the result as percentage of calcium carbonate (*a*) in the dry shell and (*b*) in the original shell.

N.B.—A small amount of the calcium in the shell is combined as calcium phosphate and is calculated as calcium carbonate in the above method.

Yolk and White. *Fat.* Weigh 3 gm. of the sample (dry matter), place in a thimble in a Soxhlet extractor and complete the determination as on p. 93. Calculate the results as percentages (*a*) in the dry matter, and (*b*) in the original yolk and white respectively.

Note the small percentage of fat in the white, and the high percentage of material soluble in petroleum ether in the yolk. This in addition to fat will include lecithin and cholesterol which are soluble in petroleum ether.

Protein. Accurately weigh about 0·5 gm. of the dried white, and about 1 gm. of the dried yolk, and transfer to Kjeldahl flasks. Complete the determination as described for total nitrogen in soils (p. 28), using about 75 ml. of 0·1N. acid in the receiving flask in the case of the white, and 50 ml. of 0·1N. acid in the case of the yolk. To obtain the percentage of protein, multiply the percentage of nitrogen by 6·7[1] for egg white and 6·62[1] for yolk. Express the results as percentages (*a*) in the dry matter, and (*b*) in the original yolk and white respectively.

[1] According to Plimmer.

Ash. Accurately weigh about 3 gm. in a weighed dish and ignite until a greyish white ash is obtained. Cool and weigh. Express the results as percentages (*a*) in the dry matter, and (*b*) in the original yolk and white respectively.

Analysis of Whole Egg. From the results obtained for shell, yolk and white respectively, calculate the percentages of water, protein, oil and ash in the original egg.

FEATHERS

Examination of Feathers. Cut up some feathers very finely with scissors and examine as follows :

Sulphur. Place a mixture of equal parts of potassium nitrate and potassium carbonate in a crucible and heat until fused. While fused add some of the feathers in small portions at a time, and heat until completely oxidised. Cool and dissolve the residue with dilute hydrochloric acid. Add barium chloride solution and note the white precipitate of barium sulphate, indicating the presence of sulphate formed from the sulphur of the feathers.

N.B.—Feathers consist largely of proteins called keratins which are rich in sulphur.

Moisture and Ash. Accurately weigh about 2 gm. of feathers, and dry in an oven at 100° C. until constant in weight. The loss in weight represents the moisture present. Ignite the residue until a greyish white ash is left, cool and weigh. Calculate the percentage of moisture and of ash.

Protein. Accurately weigh about 0·5 gm. of feathers, transfer to a Kjeldahl flask, and carry out the determination as described under egg white. To obtain the percentage of protein multiply the percentage of nitrogen by 6·25.

CHAPTER IV

FEEDING STUFFS

THE feeding stuffs used for stock vary considerably in composition and contain a large number of different substances, some of which are only present in traces. In the ordinary chemical analysis of feeding stuffs, some or all of the following determinations are carried out, although under the regulations of the Fertilisers and Feeding Stuffs Act, only the contents of one, two or three of the main constituents are required to be stated. These determinations are (1) Water, (2) Crude Protein or Albuminoids, (3) Crude Fat, Oil or Ether Extract, (4) Soluble Carbohydrates or Nitrogen-free Extract, (5) Crude Fibre, (6) Ash.

In some cases other determinations are required, such as the percentage of sand, salt or sugar present, while in herbage and fodder crops generally the contents of different minerals present are particularly important. In certain crops, especially root crops, the proportion of non-protein nitrogenous substances is very high, and as these substances have a lower feeding value than proteins, it may be necessary to distinguish between " crude " and " true " protein. Some materials are used in feeding because of their high vitamin content and should conform to certain standards. A few feeding stuffs contain cyanogenetic glucosides and may cause prussic acid poisoning ; in such cases the determination of the prussic acid may be important. A knowledge of the digestibility of the constituents of feeding stuffs affords more valuable information than chemical composition, and the determination of the digestibility of the protein can be carried out in the laboratory. This section is completed with leaf analyses used in the detection of mineral deficiencies.

Preparation of the Sample for Analysis. The sample is ground to

a fine condition so that it will pass through a sieve having apertures 1 mm. square, thoroughly mixed and about 100 gm. placed in a stoppered bottle, from which portions required for the analytical determinations are taken. If the original sample is appreciably moist, or may lose or gain moisture in the pulverisation and mixing, the moisture content of the original sample and of the powdered sample should be determined. The results of the analysis are then corrected to the original moisture content. In the case of root crops special sampling methods are used.

ANALYSIS OF FEEDING STUFFS

The student is advised to carry out the following determinations with feeding stuffs of varying types, *e.g.* a coarse fodder, a cereal grain and an oil cake.

Determination of Moisture. Accurately weigh from 2 to 5 gm. of the sample in a weighing bottle and dry to constant weight in an oven at 100° C. The loss in weight is taken as moisture.

Determination of Crude Protein. This determination is based on the assumption that the nitrogenous compounds in feeding stuffs contain 16 per cent. of nitrogen. The percentage of nitrogen multiplied by the factor 6·25 is stated as crude protein (albuminoids).

Accurately weigh 1 gm. of the sample and transfer into a Kjeldahl flask, add 20 ml. of concentrated sulphuric acid, 10 gm. of powdered potassium sulphate and a small crystal of copper sulphate. Proceed with the digestion as on p. 28 and continue to heat for about half an hour after the contents of the flask are clear. The determination is now completed as described on p. 29, using from 25 to 100 ml. of 0·1N. sulphuric acid in the receiving flask. Calculate the percentage of crude protein by multiplying the percentage of nitrogen by 6·25.

Determination of Crude Fat, Oil or Ether Extract. Accurately weigh 2 or 3 gm. of the sample and place in an extraction thimble. Weigh the flask of a fat-extraction apparatus, place the thimble in a Soxhlet extractor and attach the weighed flask. Pour sufficient petroleum ether (boiling point 40° to 60° C.) into the extractor to start the siphon and then half fill the extractor again. Attach the extractor to the condenser and heat the flask on a water bath or

electric hot plate for six to eight hours. When the extraction is complete, remove the thimble and distil the petroleum ether into the extractor. Remove the flask, place in an oven at 100° C. to drive off the last traces of the solvent and dry to constant weight. The increase in weight of the flask represents the crude fat or oil, although it will also include such substances as waxes, resins or colouring matters if these were present in the feeding stuff.

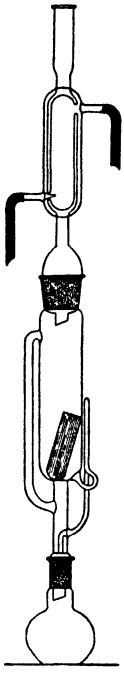


FIG. 9.—Soxhlet extraction apparatus.

N.B.—In the case of feeding stuffs containing saccharine matter, the weighed portion in the thimble is washed with water and then dried previous to the extraction.

Determination of Crude Fibre. This is most conveniently carried out on the fat-free residue in the thimble from the previous experiment. It is a conventional method, giving comparable results only when the prescribed conditions are rigidly observed. It represents the fraction of the feeding stuff (less insoluble sand) which is insoluble in sulphuric acid and sodium hydroxide solutions of 1·25 per cent. concentration, when boiled for a prescribed time (30 minutes). It consists mainly of the celluloses of the feeding stuff, *i.e.* pure cellulose and the compound celluloses such as ligno- and cuto-cellulose.

Dry the thimble and contents used in the fat determination and transfer the residue to a 400 ml. beaker, which has been previously marked at the 200 ml. level. Add 50 ml. of 5 per cent. sulphuric acid solution and make up to the 200 ml. mark with distilled water. Bring the contents of the beaker to the boil and keep just boiling for exactly 30 minutes. During the boiling stir with a rubber-tipped glass rod, removing all particles from the sides, and keep the volume constant by the addition of hot water from time to time. Filter immediately through linen on a Buchner funnel attached to a filter pump. Transfer the residue to the funnel with a jet of hot water and wash with hot water until the filtrate is free from acid. Scrape off the residue from the linen with a spatula and replace in the same beaker, removing the last traces with a jet of hot water. Add 50

ml. of 5 per cent. sodium hydroxide solution and make up to the mark with distilled water. Bring to the boil, keep boiling for exactly 30 minutes, stir with a rubber-tipped glass rod and keep the volume constant by the addition of hot water at intervals. Filter immediately through the same linen and transfer the residue to the filter by means of a jet of hot water. Wash the residue a few times with hot water, then with a solution of 1 per cent. hydrochloric acid and again with boiling water until free from acid. Finally wash twice with a little 95 per cent. alcohol, transfer the residue to a porcelain dish, removing the last traces with a jet of alcohol.

Evaporate to dryness and dry in an oven at 100° C. until constant in weight. Cool in a desiccator and weigh the dish and contents. Ignite at a dull red heat until only a little grey ash is left, cool and weigh again. The loss in weight represents the crude fibre.

Determination of Ash. Accurately weigh from 2 to 5 gm. of the sample into a weighed crucible and ignite. Keep the flame low at first, stir with a fine wire, gradually increase the flame and heat until a greyish-white ash is left. Cool in a desiccator and weigh.

Determination of Soluble Carbohydrates or Nitrogen-free Extract. This is not a direct determination, but is obtained by adding together the percentages of the other constituents and subtracting the total from 100.

These consist chiefly of starches and sugars, but other carbohydrates such as dextrins, gums, mucilages and pectic substances, if present in the feeding stuff, will be included.

Determination of True or Pure Protein. The nitrogenous compounds of feeding stuffs, apart from proteins, include amides, amino acids and perhaps ammonium salts and nitrates. In root crops and green crops particularly, a high proportion of the nitrogen is present in these forms. The non-protein nitrogenous matter is assumed to have half the nutritive value of true proteins, therefore the determination of the true protein as distinct from the crude protein is of importance.

In the following method for determining true protein, advantage is taken of the fact that the non-protein nitrogenous substances are soluble in water and that soluble proteins are precipitated by certain metallic salts, *e.g.* cupric hydroxide.

Weigh 1 gm. of the foodstuff, transfer to about 100 ml. of water

in a beaker, and boil. (Substances containing much starch should be heated on a water bath for ten minutes.) Stir with a glass rod, add about 5 ml. of Stutzer's reagent (a suspension of $Cu(OH)_2$) and continue to boil for about a minute. Allow the precipitate to settle, filter, using a Buchner funnel and suction pump, and thoroughly wash the precipitate with hot water. (The precipitate contains the true proteins of the feeding stuff.) Transfer the paper and precipitate to a Kjeldahl flask, add 25 ml. of concentrated sulphuric acid, 10 gm. of potassium sulphate and complete the determination as described under crude protein.

The non-protein nitrogenous material is obtained by subtracting the percentage of pure protein from the percentage of crude protein.

Determination of Sand or Siliceous Matter. If the percentage of ash in a feeding stuff is unduly high, it is often important to determine the nature of the mineral matter present. In particular, the high ash content may be due to a high percentage of sand and give rise to injury when fed to animals. In fodders such as grass or hay, the sand content is often determined to obtain the silica-free ash content, which gives a better indication of the useful minerals present than does the total ash figure.

Weigh from 2 to 5 gm. of the sample into a small weighed evaporating dish and incinerate to obtain the ash. Moisten the ash with concentrated hydrochloric acid and evaporate to dryness. Extract the ash several times with hot dilute hydrochloric acid, filter, transfer the insoluble matter to the filter paper and wash with hot water. Replace the filter and residue in the dish, dry and ignite. Cool and weigh ; the increase in weight is taken as sand or siliceous matter.

N.B.—The filtrate may be tested qualitatively for the presence of calcium, potassium and phosphate.

MINERAL INGREDIENTS IN FEEDING STUFFS

The importance of mineral ingredients in feeding stuffs is now fully recognised. As a general rule on the farm, animals obtain most of their "natural" mineral matter from herbage plants. In particular, the mineral composition of pastures and of individual pasture grasses and clovers has been largely in-

vestigated. In the case of such plants, the proportion of certain minerals present may be as important from a feeding point of view as their contents of protein, oil and carbohydrates. Certain feeding stuffs such as fish meal and meat and bone meal are also rich in mineral matter. Methods are given here for the determination of three of the more important minerals which are most likely to be deficient in the ordinary rations of farm animals.

Determination of Calcium. Accurately weigh from 2 to 5 gm. of a sample of dried grass (or other fodder), transfer to a porcelain dish and ignite until a grey ash is obtained. Extract the ash four times with hot dilute hydrochloric acid and filter. Transfer the residue with a jet of hot water to the filter paper and thoroughly wash several times with hot water. Add distilled water to bring the volume of the filtrate to about 100 ml., make just alkaline with ammonia and add sufficient acetic acid to redissolve the precipitate. Proceed with the determination as described on p. 17 and express the result as percentage of calcium oxide (CaO).

This method may be used for any feeding stuff; in the case of the oil cakes and cereal grains which have a low content of calcium, about 10 gm. should be taken; for fish meal or meat and bone meal, weigh 2 gm., make up the extract to 500 ml. and use 100 ml. for the determination.

Determination of Phosphorus. Accurately weigh about 2 gm. of the sample and transfer to a Kjeldahl flask. Add 20 ml. of concentrated sulphuric acid and 10 ml. of concentrated nitric acid. Heat over a low flame until all the nitric acid fumes have disappeared. If the contents are still brown add a further 10 ml. of concentrated nitric acid and heat again. These additions of nitric acid should be repeated until the contents of the flask are completely clear, showing that all the organic matter is destroyed and the phosphoric acid is completely in solution. Allow to cool, dilute the contents and wash out into a 400 ml. beaker. Slowly add ammonia until slightly alkaline, and reacidify with nitric acid. The determination is now completed as described on p. 49 and the result is expressed as percentage of "phosphoric acid" (P_2O_5).

Determination of Chlorine. (*a*) *By Wet Oxidation Method.* This method is suitable for fodders and for those feeding stuffs containing a comparatively low percentage of protein. **For**

feeding stuffs rich in protein, *e.g.* fish meal or meat meal, the solution obtained is highly coloured and it is difficult to obtain a sharp end point in the titration. In the case of such feeding stuffs the second method described gives more accurate results.

Accurately weigh 1 or 2 gm. of the sample and transfer to a conical flask. Add sufficient distilled water to cover the sample, 20 ml. of 0·05N. silver nitrate solution, about 10 ml. of a saturated solution of potassium permanganate and about 25 ml. of pure concentrated nitric acid. Boil over a gauze until the liquid is clear ; this generally takes about five minutes. Cool, dilute the contents to about 100 ml. and add about 25 ml. of acetone (this gives a sharper end point in the subsequent titration). Add 1 ml. of a saturated solution of iron alum in 10 per cent. nitric acid and titrate the excess silver nitrate with 0·05N. potassium thiocyanate solution, *i.e.* until the reddish tinge remains permanent.

N.B.—If it is found that the silver nitrate is not in excess, the determination must be repeated, using a larger volume of the silver nitrate solution.

The equations for the above reactions are :

(1) $NaCl + AgNO_3 = AgCl + NaNO_3$.

(2) $AgNO_3 + KCNS = AgCNS + KNO_3$.

(3) $6KCNS + Fe_2(SO_4)_3 = 3K_2SO_4 + 2Fe(CNS)_3$.

Calculate the percentage of chlorine present and express as chloride (Cl).

1 ml. of 0·05N. $AgNO_3 = 0·001775$ gm. Cl.

(*b*) *By ashing with Calcium Oxide.* This method is advised for fish meal, meat meal, or meat and bone meal and for the oil cakes rich in protein, such as decorticated ground nut cake or soya bean cake.

Accurately weigh 2·5 gm. of the sample into a porcelain dish, cover with about an equal quantity of pure calcium oxide and make into a paste with a little distilled water. Dry on a sand bath and then ignite over a low flame until the organic matter burns away. Extract the residue four or five times with hot dilute nitric acid, filter and collect the filtrate in a 250 ml. graduated flask. Transfer the residue to the filter paper with a jet of distilled water and thoroughly wash with hot distilled water. Add 20 ml. of 0.1N. silver nitrate solution to the contents of the flask, shake and allow the precipitated silver chloride to settle out in the dark. Make up

to the mark with distilled water, shake well to mix the contents, and filter through a large dry folded filter paper, collecting the filtrate in a dry beaker. Pipette 100 ml. of the filtrate into a conical flask, add 1 ml. of iron alum solution as indicator, and titrate the excess silver nitrate with 0·1N. potassium thiocyanate solution.

The reactions are the same as in the previous method. In the case of fish meal, meat meal or meat and bone meal, the result should be stated as percentage of sodium chloride (NaCl).

1 ml. of 0·1N. $AgNO_3 = 0·00585$ gm. NaCl.

ROOTS, SUGAR BEET AND MANGOLDS

These crops, particularly sugar beet, are characterised by containing a high percentage of cane sugar, and the crude protein includes a large proportion of non-protein nitrogenous substances, among which nitrates are present. The percentage of sugar in sugar beet is important, as this crop is used as a commercial source of cane sugar.

General Analysis of Sugar Beet or Mangolds. *Sampling.* To obtain reliable results, fifty roots chosen by random selection should be taken.

The sample for analysis may be obtained in a number of ways: (1) cores are removed from each root and then put through a mincer; (2) a section taken lengthwise from crown to tail is obtained with a circular saw, the resulting pulp being collected in a hopper below; (3) a fine pulp is obtained by means of a conical rasp. The rasp revolves on a central spindle, the beet is pressed on the top edge of the rasp which cuts out a wedge-shaped sector from the tail to the crown of the beet. A finer pulp is obtained in methods (2) and (3) than in the first method. The pulp from all of the beets sampled is then thoroughly mixed, and portions weighed out for the analytical determinations, which should be carried out in duplicate.

Determination of Water. Weigh 20 gm. of the pulp into a weighed dish and place in an air oven at 50° C. to 60° C. for about two days. Transfer to an oven at 100° C. and dry for a further period of six hours.

Determination of Crude Protein. This is determined on air-dried pulp which has been finely ground. At the same time a moisture determination is carried out on 2 gm. of the air-dry sample so that the result can be recalculated to the original moisture content of the roots. Since nitrates are often present, a modification of the Kjeldahl method is employed, salicylic acid being added which fixes the nitrate nitrogen as nitro-salicylic acid, a compound which readily undergoes reduction with sodium thiosulphate to form an amino acid. The amino acid then undergoes change to ammonia as in the usual Kjeldahl method.

Accurately weigh about 2 gm. of the air-dry material and transfer to a Kjeldahl flask. Add 30 ml. of concentrated sulphuric acid containing 1 gm. of salicylic acid, and shake the flask immediately to mix the contents without delay. Keep the flask cool, and after shaking for about ten minutes add 10 gm. of potassium sulphate, 5 gm. of crystalline sodium thiosulphate and a small crystal of copper sulphate. Heat the flask in a fume chamber until the contents are clear, and for a further period of about half an hour. The determination is now completed as on p. 29. Multiply the percentage of nitrogen by 6·25 to obtain the percentage of crude protein.

Determination of True Protein. This is determined as described on p. 95. In these crops the amount of true protein may be less than half that of the crude protein.

Special Determinations in Sugar Beet. The value of sugar beet as a source of sugar depends on two factors, the purity of the juice and the percentage of cane sugar present.

Determination of Total Solids in Juice. The purity of the juice, or the " coefficient " or " quotient of purity ", is the percentage of sugar in the total solids of the juice.

The percentage of sugar in the juice can be obtained by multiplying the percentage of sugar in the root by a factor. This factor is a correction for the amount of " marc " or cellular matter present in the beet. Beet is usually taken as containing 95 per cent. juice and 5 per cent. " marc ", therefore the factor is 100/95.

Place some pulp in a linen cloth and express the juice by means

of a press. Allow the juice to stand for one hour in order to remove all air bubbles, and then transfer to a tall cylinder. Add a few drops of ether to destroy the surface froth and float a Brix hydrometer in the juice. Take the reading of the instrument which is graduated to give directly percentages of the solids in solution. Correct the readings for temperature if necessary by means of the scale on the bulb.

Determination of Sugar. Two methods are given for the extraction of the sugar from the pulp : (*a*) cold water extraction, (*b*) hot water extraction. The cold water extraction is reliable where a fine pulp is obtained by the use of a saw or rasp. If the pulp is rather coarse, it is better to use the hot water extraction to ensure that all of the sugar diffuses into the water.

(1) *By Polarimeter.* The determination is carried out using a polarimeter which is graduated in sugar degrees, *i.e.* the percentage of cane sugar can be read off without calculation when the normal weight (p. 137) of pulp is used. In the determination of cane sugar the normal weight for most instruments is 26 gm.

Hot Water Extraction. Weigh 26 gm. (normal weight) of well mixed pulp and transfer to a 200·6 ml. Kohlrausch wide-mouth flask. Add 5 ml. of basic lead acetate solution, fill up to within one inch of the mark with hot water and immerse in a water bath at 80° C. to 85° C. for half an hour. The basic lead acetate is a clarifying agent and precipitates proteins and other optically active substances. Cool to 20° C., add a few drops of ether to dispel the froth, and make up to the mark with distilled water. Thoroughly mix and filter through a dry filter paper into a dry beaker and polarise (p. 137) in a 200 mm. tube. The reading multiplied by two is the percentage of cane sugar. The reading has to be multiplied by two because the normal weight was made up to 200 ml.

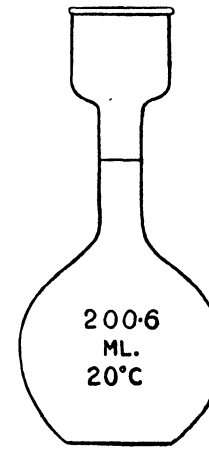


FIG. 10.—Kohlrausch flask.

Cold Water Extraction. Weigh 26 gm. of well mixed pulp and transfer to a beaker. Add 5 ml. of basic lead acetate solution, 172 ml. of water and stir thoroughly for five minutes. Filter through a dry filter paper and polarise the filtrate in a 200 mm. tube as above.

The 172 ml. of water with 5 ml. of basic lead acetate are added to make the volume of the final solution 200 ml., for it is assumed that 26 gm. of pulp will contain 23 ml. of juice.

N.B.—If the polarimeter available is not graduated in sugar degrees, take the reading in angular degrees and compare with the reading of a sugar solution made by dissolving 26 gm. of pure cane sugar in water and making up to 200 ml.

(2) *By Titration with Benedict's Solution.* For titration with Benedict's solution the cane sugar must first be hydrolysed to invert sugar, and this is best effected by means of 10 per cent. citric acid. In this method the red precipitate of cuprous oxide (obtained when Fehling's solution is used and which obscures the end point) combines with potassium thiocyanate present in the reagent to form an insoluble white compound. The usual Benedict's method is improved by the use of methylene blue as an internal indicator. Benedict's solution is of such strength that 25 ml. = 0·05 gm. invert sugar or 0·047 gm. cane sugar, but for accurate work the solution should be standardised with pure cane sugar.

Weigh 25 gm. of well mixed pulp, transfer to a beaker, add 5 ml. of basic lead acetate solution and about 150 ml. of hot water. Place in a water bath at about 80° C. for half an hour. Add sufficient sodium carbonate (about 1 gm.) to precipitate the excess lead acetate, and filter through a large folded filter paper, collecting the filtrate in a 500 ml. flask. Transfer the pulp to the filter and thoroughly wash with hot water. Cool the filtrate, make up to the mark with distilled water and well mix the contents.

Pipette 25 ml. into a small conical flask, acidify with one drop of concentrated sulphuric acid, dilute to 50 ml. with distilled water and add 5 gm. of citric acid to make the concentration of the latter 10 per cent. Boil for ten minutes, cool and neutralise with sodium hydroxide, using a drop of phenolphthalein as indicator. Transfer to a 100 ml. graduated flask, rinse with distilled water and make up to the mark.

Place the sugar solution in a burette. Pipette 25 ml. of Benedict's solution into a 250 ml. conical flask. Add 4 gm. of anhydrous sodium carbonate and a few pieces of porous pot to prevent bumping. Bring to the boil, and while vigorously boiling run in the sugar

solution (0·5 ml. increments) at intervals of ten seconds, until the blue colour practically disappears. Add two drops of 1 per cent. solution of methylene blue, boil vigorously, and add the sugar solution drop by drop every fifteen seconds until colourless. The end point should be quite sharp.

If the mixture becomes too concentrated during the titration, add boiling water from time to time to replace that lost by evaporation.

Standardisation of Benedict's Solution. Accurately weigh 4·75 gm. of pure (analytical quality) sucrose, dissolve in distilled water, transfer to a 500 ml. graduated flask and make up to the mark with distilled water. Pipette 20 ml. into a small conical flask, acidify with one drop of sulphuric acid, dilute to 50 ml. with distilled water and add 5 gm. of citric acid. Boil as above, neutralise, cool and dilute to 100 ml. in a graduated flask. 25 ml. of this solution = 0·05 gm. invert sugar or 0·0475 gm. cane sugar. Place this solution in a burette and titrate as above with 25 ml. of Benedict's solution.

Example. Suppose 25 ml. of Benedict's solution required 24·6 ml. of the standard sugar solution.

∴ 25 ml. of Benedict's solution are equivalent to

$$0{\cdot}0475 \times 24{\cdot}6/25 = 0{\cdot}04674 \text{ gm. sucrose.}$$

Suppose 20·6 ml. of hydrolysed beet extract were used by 25 ml. of Benedict's solution.

∴ 100 ml. of hydrolysed beet extract

$$= 0{\cdot}04674 \times 100/20{\cdot}6 = 0{\cdot}228 \text{ gm. sucrose.}$$

This is equivalent to 1/20 of the total sugar in 25 gm. of beet, since 25 ml. were taken for hydrolysis, out of a total of 500 ml. of extract.

∴ 25 gm. of beet contain $0{\cdot}228 \times 20$ gm. sucrose.

Percentage of sucrose $= 0{\cdot}228 \times 20 \times 100/25 = 18{\cdot}24$.

"Noxious Nitrogen." In addition to sugar and cellular matter sugar beet contains varying amounts of nitrogenous substances other than proteins, *e.g.* amino acids etc. which make it difficult for the factory to obtain a complete yield of crystallisable sugar.

These substances are accordingly classed as "noxious nitrogen" the amounts of which are measured by the factory as a matter of routine by the following rapid method, which depends upon the determination of the depth of blue colour produced when a special copper reagent is added to a clarified water

extract of sugar beet. The colours are compared with those obtained in the same way from solutions containing known quantities of glutamine or other similar substance. The figure obtained is generally related to the conditions of soil and climate under which the beet was grown.

Dissolve 1·57 gm. of mono-sodium glutamate in water and make up to 100 ml. Dilute 2 ml. of this solution to 100 ml. and add 10 ml. of the special copper reagent (2·5 gm. copper acetate and 62·5 gm. sodium acetate dissolved in *cold* water and make up to 250 ml.). This will give a blue standard colour equivalent to 20 mgm. of nitrogen per 100 gm. of beet. In a similar manner prepare a series of standard colours representing 0 to 100 mgm. of nitrogen per 100 gm. of beet, by steps of 20 mgm. In certain cases the standards should extend from 0 to 200 mgm. Pour the standards into flat bottomed specimen tubes of approximate size 6 in. × ½ in. and marked at a volume of 22 ml.

To 20 ml. of freshly filtered clarified extract from the cold water extraction method given above, add 2 ml. of the special copper reagent, mix and pour into a specimen tube similar to that used for the standards. Match its colour immediately against that of the nearest standard by placing the tubes upon a white tile or piece of milk glass and view through the column of liquid.

" FREE " PRUSSIC ACID IN LINSEED CAKE

Linseed and linseed cake contain a cyanogenetic glucoside (p. 83) which on hydrolysis yields prussic acid. The total amount of prussic acid present can be determined by extracting the glucoside with hot alcohol, and distilling off the prussic acid after the addition of sulphuric acid. A better guide to the possible amount of prussic acid which may be formed, when linseed cake is used as a feeding stuff, is the amount of prussic acid obtained when the cake is macerated with water and allowed to stand at blood heat. This is termed " free " prussic acid.

Place about 250 ml. of water in a round-bottomed flask of about 1½ litres capacity, warm to 38° C. and add a few drops of toluene to keep the contents aseptic. Add a weighed quantity of the finely

ground cake (25 or 50 gm.), mix the contents, close with a rubber stopper and place in a thermostat or incubator maintained at 38° C. After twelve hours remove the flask and steam distil the contents for about half an hour into 50 ml. of saturated sodium bicarbonate solution. Titrate the distillate with 0·02N. iodine solution, using starch solution as indicator. The iodine reacts according to the following equation.

$$HCN + I_2 = CNI + HI$$

$\therefore$ 1 ml. of 0·02N. iodine = 0·00027 gm. HCN.

The purpose of the bicarbonate is to combine with the hydrogen iodide formed, so that the reaction can go to completion.

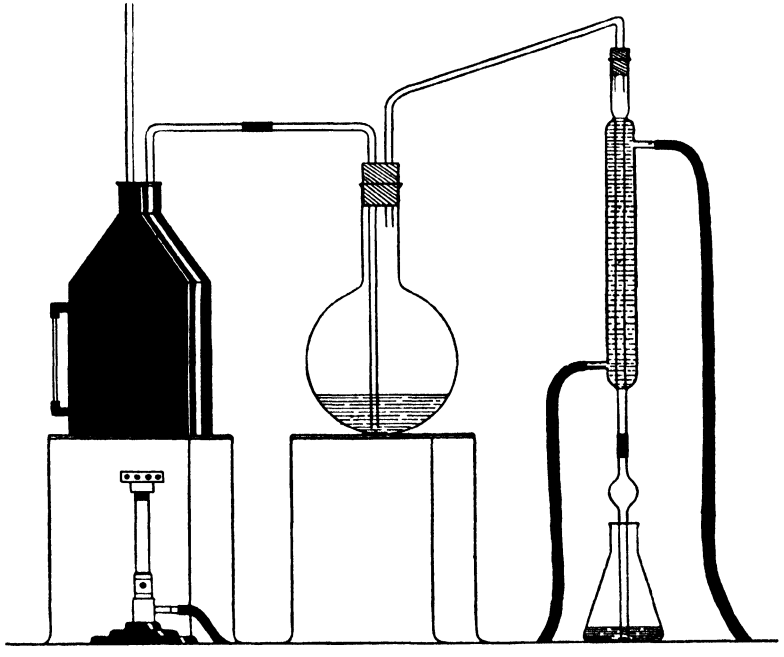


FIG. 11.—Apparatus for distillation with steam.

MISCELLANEOUS PRODUCTS USED IN ANIMAL FEEDING

The normal rations of farm animals are sometimes deficient in either mineral matter or vitamins or both. Various substances are used to make good these deficiencies. The most important of the mineral substances used are common salt, which supplies both sodium and chlorine, sterilised steamed

bone flour to supply phosphorus, ground chalk, limestone grit or oyster shell to supply calcium. To supply vitamins A and D, cod liver oil is very largely used, while dried yeast can be used to supply vitamin B.

Determination of Calcium Carbonate in Ground Chalk, Limestone Grit or Oyster Shell. The value of all of these materials depends on their content of calcium carbonate. The limestone grit or oyster shell should be finely ground before a portion is weighed for analysis.

Accurately weigh about 2·5 gm. of the sample in a porcelain dish. Ignite to get rid of any organic matter if present. Extract the ash four times with hot dilute hydrochloric acid, filter into a 500 ml. graduated flask and thoroughly wash the filter with hot distilled water. Cool the filtrate, make up to the mark with distilled water and shake well. Pipette 25 ml. into a beaker, make slightly alkaline with ammonia and reacidify with acetic acid. Add distilled water to make the volume about 100 ml., proceed to determine the calcium present (p. 17) and calculate the rest as calcium carbonate ($CaCO_3$).

1 ml. of 0·1N. $KMnO_4 = 0·005$ gm. $CaCO_3$.

Determination of Phosphoric Acid in Steamed Bone Flour. Accurately weigh 2 gm. of the sample, transfer to a Kjeldahl flask and digest with concentrated sulphuric and nitric acids (p. 97) until clear. Cool, dilute the contents with water, filter into a 500 ml. graduated flask and thoroughly wash the insoluble matter with water. Make up the filtrate to the mark with water, shake well and determine the phosphoric acid in 100 ml. of the solution.

N.B.—This method is also suitable for fish meal or meat and bone meal, but a larger aliquot of the filtrate should be used.

Cod Liver Oil. Cod liver oil varies considerably in quality. In the production of the better grades the oil is extracted from fresh (under one hour old) livers, by means of high pressure steam. The oil so obtained is light-coloured, practically odourless and tasteless, with a free fatty acid content of under one per cent. In some processes the livers are allowed to rot, and in course of rotting release their oil, which is generally reddish brown or dark red, with an objectionable flavour, nauseating smell and high free fatty acid content.

Determination of Free Fatty Acid. Accurately weigh a small dish containing about 10 gm. of the oil. Pour into a conical flask and reweigh the dish, thus obtaining the weight of oil taken. Add about 100 ml. of hot neutral alcohol and a few drops of phenolphthalein and shake vigorously. Titrate with 0·1N. sodium hydroxide solution with constant shaking until the pink colour remains permanent. From the number of ml. of 0·1N. alkali used, calculate the percentage of acid present, stating the result in terms of oleic acid.

From the equation

$$C_{17}H_{33}COOH + NaOH = C_{17}H_{33}COONa + H_2O.$$

1 ml. of 0·1N. NaOH = 0·0282 gm. oleic acid.

Medicinal oils for human consumption should contain not more than 0·6 per cent. free fatty acid, while good quality veterinary oils are usually guaranteed to contain under 1 per cent. of free fatty acid.

Determination of Blue Value (approximate measure of vitamin A content). Cod liver oil, when treated with antimony trichloride, gives a blue colour, and when the test is carried out under prescribed conditions, the measurement of the depth of colour produced gives the "blue value" of the oil. The blue colour is due to the presence of vitamin A in the oil, although the depth of blue colour may not be directly proportional to the vitamin A content. A good quality oil should have a minimum blue value of 6 (measured in Lovibond units), while many oils have a much higher value.

Weigh 2 gm. of the oil in a dry 10 ml. flask and fill to the mark with dry chloroform. Mix the contents, pipette 0·2 ml. into a small cell (1 cm. internal diameter) and add 2 ml. of antimony trichloride reagent (a saturated solution of $SbCl_3$ in chloroform). This reagent is best added from a bottle of the reagent fitted with an automatic pipette to deliver 2 ml. (In this way the reagent is prevented from becoming moist, and the sediment which forms in the reagent is not disturbed.) Observe the depth of blue colour produced, using a Lovibond tintometer to match the colour. The depth of colour soon reaches a maximum before fading, and the colour should be read at maximum intensity. It is advisable to

take several readings; from the first readings approximate results can be obtained so that in the final reading little adjustment of the colour standards should be necessary.

N.B.—This test may be performed qualitatively to compare different oils, by using the chloroform solution of oil and reagent in the above proportions, and noting the depth of colour produced in each case.

Determination of Unsaponifiable Matter. The vitamin A of cod liver oil is contained in the unsaponifiable matter. If the blue values of cod liver oils are determined via the unsaponifiable matter, much higher values are obtained than those determined directly on the oils, because the oils contain substances which inhibit the development of the blue colour. The proportion of unsaponifiable matter should not be unduly high, and in good quality oils the figure should not exceed 1·5 per cent.

Weigh a small beaker, add about 5 gm. of the oil and weigh again. Add 20 ml. of an approximately 2N. alcoholic potassium hydroxide solution and heat on a water bath with continual agitation for about three minutes to saponify the oil. Before the mixture is quite cold add 50 ml. of ether and pour into a separating funnel. Shake the latter to mix the contents, allow to settle and run off the bottom soapy layer into another separating funnel. Add 50 ml. of ether to the latter, shake and allow to settle. Withdraw the bottom aqueous layer and transfer the ether layer to the original funnel. Wash the contents three times with about 25 ml. portions of water and transfer the ethereal solution to a small weighed beaker. Rinse the funnel with a few ml. of ether and add to the beaker. Evaporate to dryness on a water bath, add a little alcohol to the residue and evaporate again (this frees the residue from water), and finally dry in an oven at 100° C. for fifteen minutes. Cool in a desiccator and weigh, the increase in weight being the unsaponifiable matter.

N.B.—The unsaponifiable matter may be shown to be rich in vitamin A by testing qualitatively for the vitamin as follows. Dissolve the residue in 25 ml. of chloroform, pipette 0·2 ml. into a dry test tube and add 10 ml. of chloroform. Pipette 0·2 ml. of this diluted solution into another test tube and add 2 ml. of antimony trichloride reagent. Note the deep blue coloration obtained.

DIGESTIBILITY OF PROTEIN IN FEEDING STUFFS

The percentage digestibility of feeding stuffs, or of the constituents of feeding stuffs, can, as a rule, be determined only by trials with animals, such trials being called digestion experiments. In the case of protein, however, if the food is treated with pepsin and hydrochloric acid under specified conditions, the determination of the insoluble protein which remains, agrees fairly well with the indigestible protein of the food as calculated from the dung nitrogen in a digestion experiment.

Accurately weigh 2 gm. of the finely ground sample and transfer to a flask containing 490 ml. of water containing 1 gm. of best scale pepsin. Add 10 ml. of 25 per cent. hydrochloric acid (25 gm. of strong HCl to 75 gm. H_2O) and keep at a temperature of 37° C. for twenty-four hours. Add a further 10 ml. of 25 per cent. hydrochloric acid to bring the concentration of the latter to 1 per cent., and continue the digestion at 37° C. for a further twenty-four hours.

Filter the contents of the flask, using a Buchner funnel and suction pump, and wash the residue with lukewarm water until free from chloride. Finally wash with a little alcohol and ether, and determine the nitrogen in the residue by the usual Kjeldahl method (p. 28). Determine the nitrogen content of the original feeding stuff, and calculate the coefficient of digestibility of the protein of the feeding stuff from the formula :

$$\frac{\text{Gm. of N. in food} - \text{gm. of N. in insoluble residue after digestion}}{\text{Gm. of N. in food}} \times 100.$$

DETECTION OF MINERAL DEFICIENCIES IN PLANTS BY LEAF ANALYSIS

In recent years much work has been done on the visual recognition of mineral deficiencies in plants ; also in some cases the effect of excess minerals, *e.g.* chlorides, can be recognised. In many cases the deficiency symptoms are well defined, but in others are not since they may be complicated by the effects produced by pests and diseases, mechanical injury or weather conditions. Consequently it is often essential to confirm the usual diagnosis by other means and one of the methods used

is to compare the mineral composition of the leaves, petioles or stems of suspected plants with those of normal healthy plants. Leaves to be compared should be of similar physiological age, be taken from the same part of the plant and taken at the same time from healthy and diseased plants. Also the leaves should be selected before the deficiency effects are too pronounced, *i.e.* they should be metabolically active when sampled.

No absolute maximum values can be given for the percentage amounts of the different minerals, below which it is certain that mineral deficiency effects would occur, as these vary under different conditions of soil, season, variety etc. It is therefore essential to compare the composition of leaves of healthy plants with those of diseased plants of the same crop and variety. The plants should be selected as near as possible to one another to minimise the effect of soil, etc.

Rapid approximate tests for minerals made directly on the green plant tissues are being developed for use in the field, but these are unsuitable for use by the student. The following determinations should be carried out on leaves which have been rapidly dried at 60° to 80° C. in a well ventilated oven, and then ground finely. Dry matter determinations can be carried out on the " dried " samples if absolute percentages in the dry matter are required, although for purposes of comparison of healthy and diseased plants this is not really necessary. Methods are described for the major elements, nitrogen, phosphorus, potassium, calcium, magnesium and chlorine and for the trace elements iron and manganese.

Nitrogen (N). Use 1 gm. and carry out the determination as described for crude protein on p. 93.

Phosphorus (P_2O_5). Some phosphorus may be lost in dry ashing unless certain precautions are taken. The following wet combustion process is rapid and no loss of phosphorus occurs.

Weigh about 0·5 gm. into a small Kjeldahl flask, add 10 ml. of nitric acid, 2 ml. of sulphuric acid and 1 ml. of perchloric acid (sp. gr. 1·54). Heat very gently, when a vigorous reaction with evolution of copious red fumes occurs. Remove the flask from the burner

until the reaction subsides. Continue the digestion at a low heat until all the nitric acid boils away and for 5 minutes after white fumes of sulphuric acid appear. Finally heat strongly for about 2 minutes, and allow to cool. The contents should be quite clear with no trace of carbon. (If a little carbon remains unoxidised, add a further 2 ml. of nitric acid and digest again to fuming stage.)

When cool dilute the digest with water and transfer to a 100 ml. flask. Determine the phosphorus content by the colorimetric method given on p. 40, comparing the blue colours with phosphate standards made from a stock solution containing 2 ml. of sulphuric acid per 100 ml. of solution.

Potassium (K_2O). Weigh about 2 gm. into a dish and ignite over a low flame. Extract the ash with dilute hydrochloric acid, filter and evaporate the filtrate to dryness. Ignite the residue and complete the determination as described on p. 33.

Calcium (CaO). Weigh about 2 gm. into a porcelain dish, ignite and extract the ash with dilute hydrochloric acid. Filter and determine the calcium in the filtrate as described on p. 17.

Magnesium (MgO). Concentrate the filtrate from the calcium precipitation to about 100 ml. and add 5 ml. of hydrochloric acid. Heat to boiling, add 10 ml. of 10 per cent. sodium citrate solution, 10-15 ml. of sodium phosphate (10 per cent. $Na_2HPO_4\ 12H_2O$) and neutralise the solution with dilute ammonia (1 water and 1 ammonia), and add an excess of ammonia (0·88) equal to about one third of the volume of the solution. Allow the precipitate to stand overnight, and complete the determination as described on p. 37.

Chlorides (Cl). Use 1 to 2 gm. and carry out the determination by the wet oxidation method given on p. 97.

Manganese (Mn). The chemical determination of manganese is based on its oxidation to permanganate, the colour of which is proportional to the amount of manganese present. The initial destruction of the organic matter is best carried out by the wet oxidation method described below (if dry ashing is carried out some manganese is retained in the insoluble siliceous residue of the ash). The oxidation of the manganese to permanganate is effected by means of potassium periodate. Small amounts or iron, producing coloured ferric salts, affect the colour of the permanganate and make matching difficult. This can be overcome, however, by the addition of phosphoric acid

since ferric phosphate is almost colourless in cold solution. The reaction for the oxidation to permanganic acid is shown by the following equation.

$$2MnSO_4 + 5KIO_4 + 3H_2O = 2HMnO_4 + 5KIO_3 + 2H_2SO_4$$

Digest about 5 gm. in a Kjeldahl flask as described under phosphorus, using 40 ml. of nitric acid, 5 ml. of sulphuric acid and 4 ml. of perchloric acid. After the organic matter is destroyed, heat strongly for 2-3 minutes and then allow to cool. When cool add 5 ml. of freshly prepared ammonium persulphate solution (5 per cent.), again heat to fuming and heat strongly for 5 minutes. When cool add 2 ml. of phosphoric acid (sp. gr. 1·7) and about 40 ml. of warm water. Filter and wash well with hot water. Evaporate the filtrate in an evaporating dish on a water bath until the volume is reduced to about 25 ml. Add one or two glass beads to promote even boiling and about 0·3 gm. of potassium periodate. Boil cautiously over a low flame until the colour of permanganate appears, then dilute with about 30 ml. of water and boil for a further two minutes to ensure maximum development of colour. Transfer to a 100 ml. flask, cool and make up to the mark with water.

Prepare standards from a solution containing 0·0001 gm. of manganese per ml. Pipette suitable amounts (0·5-5 ml.) into 100 ml. graduated flasks, dilute to about 50 ml., add 10 ml. of concentrated sulphuric acid and 2 ml. of phosphoric acid. Add about 0·3 gm. of potassium periodate and heat on a boiling water bath for about 5 minutes after the appearance of the permanganate colour. Cool and dilute to the mark.

Compare the colours of the test solution with the standards, using a colorimeter or Nessler cylinders, and express the results as parts per million.

Example. If 2 ml. of the standard solution of manganese matched the test solution :

Therefore Manganese (Mn) in parts per million

$$= 0{\cdot}0002 \times 1{,}000{,}000/5 = 40.$$

Iron (Fe). Great care must be taken to avoid contamination of samples with soil. The dried material should be ground with a pestle and mortar as the use of a steel mill may give rise to erroneously high results. Since most reagents contain traces of iron a blank determination should be carried out with 1 gm.

of pure sugar in place of the sample and using all reagents in similar quantities.

In the method described, ferric thiocyanate is produced by the interaction of ferric iron with potassium thiocyanate ; the red colour is extracted with amyl alcohol and is proportional to the amount of iron present. The depth of colour is affected by the acidity, therefore the amount of acid present in the test solution and standards must be the same. The equation for the reaction is,

$$Fe_2(SO_4)_3 + 6KCNS = 3K_2SO_4 + 2Fe(CNS)_3.$$

Digest 1 gm. in a Kjeldahl flask as described under phosphorus, using 10 ml. of nitric acid, 3 ml. of sulphuric acid and 2 ml. of perchloric acid. After the digest has been fuming strongly for 2-3 minutes, allow to cool and add drop by drop, 20 drops of hydrogen peroxide 20 vols. Again heat to fuming for one minute and allow to cool. Add 50 ml. of water, 3 ml. of hydrochloric acid and boil for half a minute. Cool, transfer the contents to a 100 ml. flask and make up to the mark with water.

Pipette an aliquot (10 ml. or more) into a 50 ml. stoppered measuring cylinder. Add one drop of nitric acid and dilute to 35 ml. Add 10 ml. of amyl alcohol (accurately measured) and 5 ml. of potassium thiocyanate solution (5 per cent.). Shake vigorously for about half a minute to extract the ferric thiocyanate into the amyl alcohol phase and allow to stand.

Prepare a range of standard colour solutions, containing known amounts of iron, in the following way. Pipette 5 to 25 ml. of a standard solution containing 0·000001 gm. of iron per ml. into 50 ml. stoppered cylinders. If the volume taken is less than that of the aliquot, dilute to this volume with dilute sulphuric acid (15 ml. H_2SO_4 per litre). Add one drop of nitric acid, dilute to 35 ml., and then add 10 ml. of amyl alcohol and 5 ml. of potassium thiocyanate as above. Shake vigorously and allow to stand.

In each case, that is with the test solution and the standards, pour off or pipette off the amyl alcohol layer. Compare the colours by means of a colorimeter, or compare visually in tubes of equal dimensions. Express the results as parts per 100,000.

Example. If a 10 ml. aliquot of test solution matched 7 ml. of the standard iron solution :

$\therefore$ Iron (Fe) in parts per 100,000 = 0·000007 × 100/10 × 100,000 = 7.

CHAPTER V

DAIRY PRODUCTS

MILK

IT is logical to commence the study of dairy products and the changes undergone in their preparation with a study of the milk from which they are derived. Although some dairy products are prepared from the milk of other animals, the milk of importance commercially and to the agricultural student is that secreted by the mammary gland of the cow. The factors causing variation in the composition of cow's milk, and the widely different nature of milk and the mammary secretion known as colostrum, which is obtained immediately after calving, are given in books on Dairying and on Dairy Chemistry.

Owing to the wide variation in the composition of samples of genuine milk, a statement of the average composition may be of little importance, but it should be noted that English law provides in its Food and Drugs Act that :

(*a*) Milk containing less than 8·5 per cent. of solids not fat shall be deemed to be adulterated by the addition of water unless the contrary be proved.

(*b*) Milk containing less than 3·0 per cent. of milk fat shall be deemed to be adulterated by the abstraction of fat unless the contrary be proved.

It is recognised that the composition of genuine milk may fall below these " presumptive limits ".

If the analyses of a large number of samples are averaged, we should expect some such average as that given below :

Average Composition of Cow's Milk

Water	87·35 per cent.
Fat	3·75 ,, ,,
Casein	2·65 ,, ,,
Albumin	0·30 ,, ,,
Globulin	0·10 ,, ,,
Other nitrogenous substances	0·25 ,, ,,
Lactose	4·85 ,, ,,
Ash	0·75 ,, ,,

EXAMINATION OF THE PROPERTIES OF THE CHIEF MILK CONSTITUENTS

In the scheme of qualitative separation given on p. 116, the following constituents of milk are obtained: casein, globulin, albumin, fat, lactose, ash. The tests noted below illustrate the nature of these constituents and some of their properties.

Casein. Use the moist casein prepared.

(*a*) Test its reaction to litmus paper.

(*b*) Make a thin paste by grinding some of the casein in a mortar with a little water. Pour into a boiling tube fitted with cork and delivery tube, add a little precipitated chalk, shake well and replace the cork so that the end of the delivery tube is under lime water. Note that carbon dioxide is evolved, due to the acidic nature of the casein.

(*c*) Test the solubility of the casein in (1) water, (2) very dilute ammonia, (3) lime water, (4) sodium hydroxide. In the last three instances, examine the effect of neutralising the base by adding acid.

(*d*) Heat some of the casein in a test tube with soda lime, and test the vapour for ammonia with moist litmus paper.

(*e*) Test separate portions of casein by the Xanthoproteic reaction, biuret reaction and Adamkiewicz's reaction and for the presence of phosphorus as given on pp. 76 and 77.

(*f*) Make a solution of casein in *very dilute* sodium hydroxide, add a few drops of phenolphthalein solution, and exactly neutralise with 0·1N. sulphuric acid from a burette. Add phenolphthalein solution to 2 ml. of formaldehyde and neutralise this also. Mix the solutions and add 0·1N. sodium hydroxide from a burette until neutral. Note that the addition of formaldehyde to the neutral solution produces

MILK
ISOLATION AND QUALITATIVE EXAMINATION OF ITS CHIEF CONSTITUENTS.

MILK.

CREAM AND BUTTER.

Cream.—To about 50 ml. of cream contained in a beaker, add about 25 ml. of concentrated HCl. Boil with constant stirring until the liquid is black. Transfer to a separating funnel, allow the fat to rise and then draw off the black liquid as completely as possible. Reject this liquid. Add hot water to the contents of the funnel, shake well and again separate and reject the washing water. Continue the washing until neutral to litmus. Draw off the purified **Fat** into a clean corked tube, and retain for subsequent experiments.

Butter.—Melt some naturally coloured butter (obtained from the dairy) in a test tube, allow the fat to separate and pour off about 5 ml. of the fat into another tube. Note the colour due to **Carotene**, and test for this pigment by adding a very small crystal of ferric chloride. Keep warm and observe the change in colour to bright green.

SEPARATED MILK.

To about 100 ml. of separated milk contained in a beaker, add 400 ml. of water and warm to 35° C. Add 15 ml. of 10 per cent. acetic acid and after 10 minutes add 10 ml. of N. sodium acetate solution. Stir well and allow the precipitate to settle. Decant as much as possible of the supernatant liquid to a fluted filter and after once washing the precipitate in the beaker transfer it to the filter.

Precipitate

Is **Casein.**

Dry a portion in the oven, and keep the remainder for examination of its properties.

Filtrate.

To a portion (about 10 ml.) add dilute NaOH solution until neutral to litmus paper and test with Fehling's solution. Note the presence of reducing sugar—**Lactose.**

To a similar portion apply the more specific methylamine test given on p. 62.

Heat a portion (about 100 ml.) on the waterbath and note the precipitate of heat coagulated **Albumin** and **Globulin.**

Exactly neutralise a portion (about 100 ml.) with ·5N. NaOH using bromothymol blue as indicator and add an equal volume of saturated ammonium sulphate solution. Stir well and allow to stand for at least one hour. Filter.

Precipitate.

Is **Globulin.**[1]

Filtrate.

Add 5 per cent. H_2SO_4 drop by drop and note the precipitate of **Albumin.**[2]

WHEY.

Note the colour due to the water-soluble pigment, **Riboflavin,** formerly known as Lactochrome. Boil about 250 ml. of whey with 5 ml. of 3 per cent. HCl for 15 minutes. Filter and make the filtrate neutral to litmus paper with milk of lime. Again filter, and evaporate on a waterbath to a syrupy condition. Put aside for **Lactose** to crystallise out. Crystallisation may be expedited by vigorously stirring the concentrated solution while cooling it in cold water.

WHOLE MILK.

Evaporate about 25 ml. of whole milk to dryness, and ignite the residue to obtain a pure white **Ash.** Reserve for examination of its constituents.

[1] Globulin is salted out of neutral solution when half saturated with $(NH_4)_2SO_4$.

[2] Albumin is salted out of acid solution at its isoelectric point when half saturated with $(NH_4)_2SO_4$.

acidity. This reaction is characteristic of proteins and is known as the aldehyde reaction. A quantitative method for the determination of milk proteins is based upon it (p. 136).

Albumin and Globulin. Repeat the test for phosphorus on the precipitates of albumin and of globulin obtained from milk. Note that the test with albumin is negative and that globulin contains a little phosphorus.

Fat. Use the fat prepared from the milk.

(*a*) Note the appearance and condition when cold and when melted, and that the melted fat permanently stains paper.

(*b*) Shake a few drops of the melted fat with water and with alcohol. Compare the results.

(*c*) Shake a few drops with a little ether, and with a little petroleum ether. Evaporate the solvent on a clock glass by the open window, and note that the fat is recovered unchanged.

(*d*) Vigorously shake up a few drops of the melted fat with about 10 ml. of sodium phosphate solution. Note that an emulsion forms, resembling milk in appearance. Divide this into four parts, and, using one as a standard, observe the effect of adding :

(1) Dilute hydrochloric acid. The emulsion is destroyed.

(2) Sodium hydroxide solution. The emulsion is stabilised.

(3) Calcium chloride solution. The emulsion is destroyed.

In the last case the phosphate is precipitated.

(*e*) Add about 5 ml. of the melted fat to 50 ml. of alcoholic potash in a 250 ml. conical flask. Immerse the flask in a water bath, and heat until the flask no longer smells of alcohol. Dissolve the residue of soap and glycerol in about 150 ml. of warm water and note the soapy odour.

(1) Rub some of the solution on the fingers, and note that it feels soapy.

(2) Shake about 10 ml. in a test tube and note that it lathers.

(3) To about 75 ml. of the soap solution add some strong brine to precipitate the soap. Filter, and note that the precipitate has the properties of soap.

(4) Evaporate the filtrate from (3) on the water bath. Note that a sticky residue remains. Heat this with potassium bisulphate and observe the pungent smell of acrolein—a characteristic decomposition product of glycerol.

(5) Add to the remainder of the soap solution about 50 ml. of dilute sulphuric acid. Note the precipitate, which consists

of mixed fatty acids insoluble in water. Add a little porous pot, attach the flask to a condenser and distil about 30 ml. Note the smell of the distillate and its acid reaction to litmus, which shows that some of the fatty acids are volatile.

Lactose. (*a*) Taste a little lactose, and note that it is sweet, but not so sweet as cane sugar. Observe the gritty and hard nature of the crystals.

(*b*) Dissolve about 5 gm. of lactose in 100 ml. of water. Boil about 20 ml. of the solution. Note any colour change. Add a few drops of sodium hydroxide solution and boil. The change which occurs is due to the formation of lacto-caramel.

(*c*) To about 10 ml. of the solution prepared under (*b*) add Fehling's solution and boil. Note from the red precipitate of cuprous oxide that lactose is a reducing sugar.

(*d*) Confirm the findings of (*c*) by adding some of the lactose solution to about 3 ml. ammoniacal silver nitrate solution. (Prepared by adding ammonia to silver nitrate solution until the white precipitate first formed *just* redissolves.) Immerse the test tube in boiling water, and note that the solution is reduced since metallic silver is deposited on the sides of the tube.

(*e*) To about 0·5 ml. of the solution add 5 ml. of water and 3 or 4 drops of a 5 per cent. solution of methylamine hydrochloride. Boil for half a minute and at once add 3 to 5 drops of a 20 per cent. solution of sodium hydroxide. Note the yellow colour which quickly changes to bright violet carmine (p. 62).

(*f*) To the remainder of the 5 per cent. lactose solution (about 70 ml.) add 5 ml. of butter milk starter (a culture of lactic acid bacteria), mix and titrate 20 ml. with 0·1N. sodium hydroxide, using phenolphthalein as indicator. Record the reading, and set the remainder aside in a warm place until the next laboratory period, when titrate a further 20 ml. Note that the acidity has increased due to formation of lactic acid from the lactose.

Lactic Acid. This constituent was not separated. Examine the laboratory specimen of lactic acid, and note that it is a syrupy liquid. Test a few drops in separate test tubes and observe that it is soluble in water, alcohol and ether. Test a few drops with Ufflemann's reagent.

Citric Acid. This constituent of milk was not separated.

Dissolve about 0·1 gm. of citric acid in 10 ml. of water, acidify the solution with a few drops of sulphuric acid, add 2 ml. of a 20 per

cent. solution of potassium bromide followed by potassium permanganate solution, added drop by drop and shaking after each addition, until a brown precipitate persists. Now add a freshly prepared solution of ferrous sulphate until a yellow solution containing a white precipitate of pentabromacetone is obtained.

This reaction forms the basis of a quantitative method ; the citric acid is oxidised by the permanganate solution to acetone dicarboxylic acid which then reacts with the bromide solution to give pentabromacetone.

Ash. (*a*) Dissolve a little of the ash in dilute nitric acid. Divide into two portions, test one portion for chlorides with silver nitrate solution, and the other for phosphate with ammonium molybdate.

(*b*) Dissolve some of the ash in dilute hydrochloric acid. Test separate portions for sulphate with barium chloride, for calcium with ammonia and ammonium oxalate, and for sodium and potassium by means of flame tests.

MILK ENZYMES

Catalase. Milk contains an enzyme that greatly accelerates the decomposition of hydrogen peroxide (H_2O_2) with the liberation of oxygen. There are two sources of the catalase in cow's milk, the mammary gland and bacteria, but the greater part in ordinary market milk is of bacterial origin.

Measure 50 ml. of fresh milk into a 100 ml. flask provided with a stopper and delivery tube for collection over water of any gas evolved. Warm the milk to 35° C. and add 25 ml. of 3 per cent. H_2O_2. Connect the delivery tube at once and collect the gas evolved in a large test tube. Test the gas to show that it is oxygen.

Peroxidase. Fresh milk also contains an enzyme that is capable of transferring oxygen from H_2O_2 to certain oxidisable compounds, the oxidation of which gives rise to compounds of characteristic colour.

(*a*) To 5 ml. of milk contained in a test tube add a few drops of tincture of guaiacum and a few drops 0·2 per cent. H_2O_2. The development of a blue colour indicates the presence of the enzyme.

(*b*) Repeat, using a freshly prepared 2 per cent. solution of paraphenylene-diamine instead of guaiacum. An immediate blue colour shows the presence of the enzyme.

(*c*) Repeat, using ortol and H_2O_2 as the reagents. A red colour shows the enzyme to be present.

(*d*) Repeat the above tests, using boiled milk, and note results.

Reductase. Methylene blue is reduced to methylene white by enzymes secreted by organisms which grow in milk. A test of the age or bacterial contamination, particularly by lactic streptococci, of milk, is based on this fact.

To 10 ml. of milk contained in a test tube add 0·25 ml. of the methylene blue solution provided (5 ml. saturated alcoholic solution in 200 ml.). Cork up and keep at a temperature of 38° to 40° C. Test fresh and old milk and note time taken to decolorise.

Phosphatase. An enzyme by this name is present in milk and possesses the power of hydrolysing esters of phosphoric acid with liberation of the acid and the alcohol. The presence of these in the free condition, after incubation of the milk with a phosphoric acid ester, denotes the presence of the enzyme.

The phosphatase test, originated by Kay and Graham, depends on the detection of the enzyme, phosphatase, which is always present in raw milk, but is destroyed at the temperature necessary for efficient pasteurisation. The absence of phosphatase indicates that the milk has been adequately heated, while its presence points to insufficient heating or to contamination with raw milk.

When milk containing phosphatase is incubated with disodium phenyl-phosphate, free phenol is liberated, and the quantity so liberated, which may be determined by a colorimetric method, is an approximate measure of the phosphatase present in the milk. The student is advised to carry out this test on samples of raw and pasteurised milk.

Rapid Phosphatase Test.[1] The following reagents are required:

(1) A buffer-substrate solution containing disodium phenyl-phosphate buffered with sodium barbitone. This solution must be

[1] The details do not refer to The Phosphatase Test for Heat Treated Milk as given in Ministry of Health Publication (addendum 139/Foods). The rapid test demonstrates that raw milk contains phosphatase.

freshly prepared, and for convenience it is marketed as compressed tablets. One tablet should be dissolved in 50 ml. of water.

(2) Folin and Ciocalteu's phenol reagent, which must be diluted with twice its volume of water before use.

It is important that this reagent should be carefully protected from contact with dust and any reducing substance.

(3) A 14 per cent. solution of very pure anhydrous sodium carbonate.

When the test is carried out under standardised conditions, a pale blue colour is produced which in the absence of phosphatase does not exceed a certain value. For the purpose of comparison, a 13 mm. tube is used to contain the test solutions, and a standard 2·3 Lovibond blue glass is provided for the measurement of the maximum permissible depth of colour. For convenience in carrying out the test, a small comparator has been designed to carry the standard blue glass and a space for the test tube. This is known as the " B.D.H. Lovibond Limitester."

Method of Performing the Test. (1) To 10 ml. of the buffer-substrate solution contained in a 25 ml. test tube add 0·5 ml. of the milk, and incubate the mixture in a water bath at 47° C. for ten minutes.

(2) Remove the tube, and cool to 15°-20° C. by immersing in cold water.

(3) Add 4·5 ml. of the diluted Folin and Ciocalteu's reagent, allow to stand for three minutes and filter.

(4) To 10 ml. of the filtrate add 2 ml. of the sodium carbonate solution, mix and place the tube in boiling water for five minutes and again filter.

(5) Compare the colour of the filtrate with the standard coloured glass in the B.D.H. Lovibond Limitester.

This test should be carried out in duplicate and at the same time duplicated control tests should be made in the following manner :

(1) Mix 10 ml. of the buffer-substrate solution with 4·5 ml. of the diluted Folin and Ciocalteu's reagent and 0·5 ml. of the milk, allow to stand for three minutes and filter.

(2) To 10 ml. of the filtrate, add 2 ml. of the sodium carbonate solution, mix and place the tube in boiling water for five minutes and again filter.

(3) Compare the colours of the four tubes.

Interpretation of the Results of the Test. If only a faint blue

colour develops in each of the four tubes, the milk has been heated. If the controls show more than a trace of blue colour and the reagents contain no free phenol, it is probable that a phenol-producing organism is present in the milk. This does *not* occur in pasteurised milk which has been kept at a satisfactorily low temperature following pasteurisation. With fresh, properly pasteurised milk, that is, milk which has been cooled to 55° F. immediately after pasteurisation and maintained between that temperature and 65° F. for not more than 18 hours, the control tubes should show only a trace of blue colour.

Galactase. This is a milk enzyme capable of causing the hydrolysis or digestion of milk proteins. It is found in concentrated form in separator slime.

To 100 ml. of fresh separated milk contained in a bottle, add 2½ ml. of chloroform and about 5 ml. of separator slime. Shake the bottle thoroughly and stopper tightly. Set aside in an incubator at 38° C., and examine at one day intervals for evidence of digestion.

THE QUANTITATIVE ANALYSIS OF MILK

Sampling. Owing to the tendency of cream to rise, considerable care must be taken in the sampling of milk if the analysis of the sample is to fairly represent the bulk. Where the sample is to represent the milk of one cow at a milking, no particular difficulty arises. It is important that the cow be well milked out or " stripped ", since the last drawn portions of milk or " the strippings " are of much higher fat content than the foredrawn milk. The milk can then be poured from the pail to another two or three times, and the sample withdrawn into a bottle, leaving space to allow the sample to be shaken prior to each determination. For most routine analyses, samples of about six to eight ounces are sufficiently large. In the case of churns, the milk can more conveniently be mixed by the use of the plunger to be found in dairies. For samples representing the bulk milk from a particular milking, it is obvious that the quantity of milk taken from each cow must bear some relationship to the animal's yield. If the total yield of all the cows of

the herd is equally distributed between churns of the same size, the same measured quantity can be taken from each churn to make a composite sample, which can, if necessary, be sub-sampled. Again, if the churns are unequally filled, but are of cylindrical shape, aliquot portions can be withdrawn from them by means of an open glass tube which is dipped to the bottom of the churn, the egress of milk being prevented by closing the top with the finger. The tube can then be lifted and allowed to discharge its contents into the bottle. This method cannot be used where the churns are of conical shape.

Determination of Titratable Acidity of Milk

This simple determination is extensively made in dairies with the object of following the course of lactic acid production from lactose, and so ascertaining the optimum conditions for the various cheese- and butter-making operations. It is also used to some extent as a criterion of the age of milk. There is, however, so much confusion of thought regarding what is measured that some explanation must be given. Milk freshly drawn from the cow is acid in reaction, the extent of which is largely dependent upon the stage of lactation of the animal. Little production of lactic acid has occurred at this stage, and there are other substances present to account for this " original " or " natural " acidity. These substances are principally mono-basic phosphates, caseinogen, acid citrates and carbon dioxide.

The titration does not discriminate between acidity due to these compounds and acidity due to lactic acid. Moreover, the action of the alkali used in the neutralisation brings about further changes the extent of which partly depends upon the concentration ; for example, dilution of the milk with water before titration prevents the precipitation of calcium salts which otherwise occurs, and increases the titratable acidity. A further complication is that varying results on the same sample of milk may be obtained by varying the amount of phenol-phthalein used as indicator. This is thought to be mainly due

to the colloidal constituents of milk, which are responsible for its opacity, masking the red colour of the indicator so that the change in its colour escapes detection by the eye unless sufficient red is formed.

The test is therefore an arbitrary measurement of the amount of alkali, which under the conditions prescribed is just sufficient to change colourless phenolphthalein to its red stage—a change in *p*H of approximately 6·6 (that of normal milk) to 8·3. From the strictly scientific point of view, it is absurd to record the results of the alkali titration of milk as percentages of lactic acid, and it is much better to express them as " degrees of acidity ", a value which states the number of ml. of normal alkali required for the neutralisation of the acidity of one litre of milk. The student is warned against being unconsciously encouraged to regard these measurements as being due to lactic acid by the use in many dairies of one ninth normal sodium hydroxide for the test, the underlying idea being that as the equivalent weight of lactic acid is 90, the burette readings may be expressed directly as percentages of lactic acid if 10 ml. of milk is titrated. Provided that it is realised that the measurement is not of lactic acid no harm results.

The technique of the test as now generally conducted is as follows :

Pipette 10 ml. of the milk into a small porcelain basin, add 1 ml. of phenolphthalein solution (0·5 gm. in 100 ml. of 50 per cent. alcohol) and titrate with N./9 sodium hydroxide until faintly pink. Practice is needed to decide when this stage is reached. Each ml. of the alkali corresponds to 0·1 per cent. of lactic acid or 11·1 degrees of acidity.

If the latter mode of stating the result is preferred, the titration of 10 ml. milk with 0·1N. alkali allows each 0·1 ml. of alkali required to be stated as one degree of acidity.

N.B.—The alkali solution is conveniently standardised with a ninth or tenth normal solution of potassium hydrogen phthalate prepared by dissolving the appropriate weight in distilled water and making up the volume to 250 ml. at 20° C. For N./9 this weight is 5·6728 gm., for 0·1N. it is 5·1055 gm. in 250 ml. of solution.

Determination of Specific Gravity and of Density[1]

(*a*) **Specific Gravity at 60° F.** The specific gravity of milk is mainly dependent upon the relative proportions of fat, non-fatty solids and water. For many years its determination has been considered of great value in the routine examination of milk, since in normal milk there is an approximate mathematical relationship between the specific gravity and the proportions of these constituents. Thus in large dairies it has been customary to determine the specific gravity and percentage of fat of large numbers of samples, and to calculate the content of non-fatty solids from these two values. At least twelve hours should elapse between the time of milking and of the determination, because of the slight increase in specific gravity which milk undergoes in that period—generally referred to as the Recknagel phenomenon.

In nearly all cases specific gravity is determined by a hydrometer, the scale of which covers only the limited range of specific gravities likely to be needed with milk (1·025 to 1·035). The instrument is usually called a lactometer, the scale being graduated in lactometer degrees—a degree being one unit in the third decimal place of actual specific gravity, e.g. 32·5° = specific gravity 1·0325. The method is speedy and inexpensive, but may be impracticable if the sample of milk is small; in such cases the specific gravity bottle may be used. Much misspent ingenuity has been exercised in the design of lactometers and many are useless. Great care should be taken when these instruments are acquired, and the graduations of the scale should be checked at the National Physical Laboratory, or by comparison with a tested lactometer in several samples of milk of fairly wide range of specific gravity. It is of the utmost importance to remember that lactometers only correctly indicate specific gravities at the temperature of calibration, viz. 60° F. It is preferable to ascertain the temperature of the milk with a separate thermometer rather than to use a lactometer

[1] The two methods are given because at present both are in use and there is uncertainty regarding the practical application of the latter.

incorporating a thermometer. It would be inconvenient to bring the temperature of all milk samples to 60° F. before testing, and this is avoided by calculating from the reading taken at the observed temperature the reading the lactometer would indicate at 60° F. It must, however, be fully realised that this calculation is sufficiently accurate for only a small temperature range (50°-70° F.). Usually a slide rule is used to enable the corrections to be made quickly. In Richmond's slide rule, the slide is marked with an arrow at 60° F. which is placed against the observed lactometer reading, the corrected reading is then read against the temperature at which the observation was made. If the slide rule is not available the temperature correction may be made by adding or deducting from the observed reading 0·11 lactometer degree for each Fahrenheit degree the temperature of the milk is above or below 60° F. The determinations of specific gravity, density, total solids and of fat should if possible be carried out on the same sample of milk, so that the results of the methods can be compared and in order to calculate solids not fat from the fat figures and lactometer or hydrometer figures.

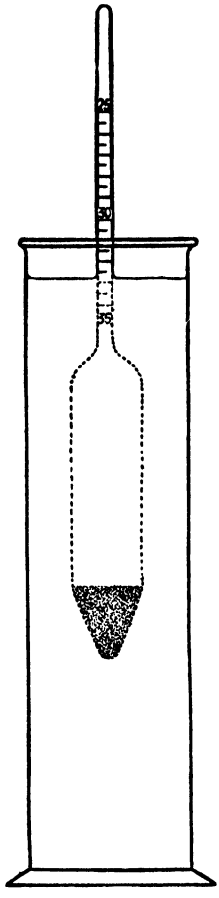


FIG. 12.—Lactometer.

Specific Gravity by Lactometer. Ascertain the specific gravity of a sample of milk by carefully immersing the lactometer in the milk which is contained in a glass or metal cylinder of suitable dimensions, the quantity of milk being sufficient to cause a slight overflow when the lactometer is inserted. Allow the lactometer to find its own level and when stationary, read on the scale the point where the surface of the milk cuts the stem. This requires practice, as this point is not visible, owing to the milk being drawn up round the stem by capillary attraction. With the majority of lactometers, the correction for this curvature is made by adding 0·5 lactometer degree

to the reading of the top of the curvature. Observe the temperature of the milk, and by Richmond's slide rule correct the lactometer reading to 60° F.

Specific Gravity by Specific Gravity Bottle. Weigh a clean, dry, specific gravity bottle, remove the stopper and fill with milk which has been brought to a temperature of 60° F. Gently insert the stopper and remove milk which has flowed on to the bottle by wiping the latter with a dry cloth (do not use the cloth to wipe the top of the stopper, but remove any milk on the top of the stopper with the finger) and again weigh. Empty the bottle and wash thoroughly with water, fill with distilled water at 60° F. and reweigh. The specific gravity is obtained by dividing the weight of milk by the weight of the water.

(*b*) **Density at 20° C.** With the object of obtaining uniformity of hydrometric measurements the British Standards Institution has designed, in a range of three sizes, a hydrometer for use in milk, the scale of which is graduated to give readings of density at 20° C. (68° F.). The density hydrometer is used in a similar manner to the lactometer described above but was intended by its designers to be used only with milk samples from which the Recknagel effect has been eliminated, by first warming the milk to 40° C. and holding it at that temperature for five minutes and subsequently cooling it to 20° C. A modified Richmond slide rule or a table of figures is needed to correct the readings to 20 °C. if these are taken at other temperatures.

Warm a sample of milk to 40° C. and maintain the temperature for five minutes, cool to 20° C. and ascertain the density at 20° C. as directed in (*a*).

Determination of Total Solids. Dry, by heating, a small flat-bottomed porcelain dish of about 7 cm. diameter, allow to cool in a desiccator and weigh. Pipette 5 ml. of the well mixed sample into it, and weigh as quickly as possible. Evaporate the milk to dryness on a water bath and dry to constant weight (four to six hours) in a steam or electric oven at 100° C. Cool in a desiccator and weigh. Calculate the percentage of total solids.

In laboratories where many determinations are made, the use of an air-damped balance results in increased accuracy and great saving of time. With such balances the operation of

weighing is conducted in a few seconds, and so little change of weight is undergone in that time that the determination can be made upon a smaller quantity of milk than is customary with balances without damping device. No increase, but actually a decrease in the percentage error of the determination, results. After cleansing in alcohol, the aluminium caps of milk bottles make convenient and extremely cheap dishes for the purpose, the variation in weight being in the majority of cases sufficiently small to render manipulation of weights unnecessary. The small sample of milk taken (about 2 ml.) speedily dries, but it is essential that the temperature of the oven used for drying shall reach 100° C. and be maintained. Electric ovens with automatic cut-out operating at this temperature are much used. An air-damped balance is not required for the method described.

Determination of Ash. Ignite the total solids from the previous determination in a muffle furnace, which should not be allowed to become hotter than a dull red heat. Cool in a desiccator and weigh. Calculate the percentage of ash.

Determination of Fat

Many methods for the determination of fat in milk are in use. A description of more than four of these would be out of place in this book. Indeed, this number would have been reduced, were it not for the fact that, as indicated later, some are better suited for use with processed milk and milk products. The methods fall into two classes, (*a*) gravimetric methods and (*b*) volumetric methods. The former are more precise, but the latter offer such great advantages of speediness and suitability for routine dairy work and milk recording in connection with livestock improvement schemes, that they are much used. The student should determine, on the same sample of milk, the fat percentage by the Rose-Gottlieb, Werner Schmidt and Gerber methods.

Gravimetric Methods. Note that the method of ascertaining the weight of milk delivered by a pipette will be found helpful in all gravimetric analyses of milk.

The Rose-Gottlieb. For liquid milk this is the best method, and is cleanly and expeditiously carried out if a tube of the type shown (Rohrig tube) is employed.

Measure with a pipette 10 ml. of the milk, and allow this to be delivered into the tube. (Ascertain the weight of this volume of milk by delivering a second quantity from the same pipette into a weighed bottle.) Add 1·5 ml. of strong ammonia (sp. gr. 0·88), shake to dissolve the caseinogen, then add 10 ml. of alcohol and again shake. Now add 25 ml. of ether and shake vigorously for one minute (release the pressure by opening the tap when the tube is inverted), and then add 25 ml. of petroleum ether and again shake for one minute. Allow to stand until the liquids have thoroughly separated and then draw off the ether layer into a Soxhlet flask. Repeat the extraction twice, collecting the ether layer in the same flask. Distil off the ether and place the flask when free from ether vapour in the steam oven, dry to constant weight. After weighing the flask, dissolve the fat by repeated extractions with petroleum ether and decant these into a bottle used for the collection of residues, allowing any sediment to remain in the flask. Dry the flask in the steam oven and weigh. By difference find the weight of fat obtained from the weight of milk taken, and calculate the fat percentage.

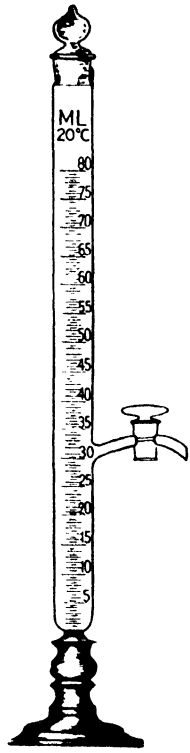


FIG. 13.—Rohrig tube.

N.B.—The reason for using petroleum ether is to prevent solution of non-fatty substances which may dissolve in ordinary ether, which is to some extent miscible with water. The alcohol breaks the ammonia emulsion and allows the ether to extract the fat.

The Werner Schmidt Method. Of the various ways in which this method may be conducted, the following is simple and efficient. Measure with a pipette 10 ml. of the milk and run it into a boiling tube about 7 in. × 1 in., which has been marked at heights corresponding to 20 ml. and 50 ml. (Ascertain the weight of this volume of milk by delivering a second quantity from the pipette into a weighed bottle.) Add 10 ml. of strong hydrochloric acid, and heat the tube on a sand bath with frequent shaking, until the contents

are dark brown, and the fat collects on the surface in a clear layer. Cool the tube under the tap and then fill the tube to the 50 ml. mark with ether. Close the tube with a good cork through which passes a glass tap to allow any pressure to be released, shake the tube vigorously and open the tap. Allow the liquids to separate, remove the cork and carefully replace it by one fitted with wash bottle tubes. Blow over the ether layer into a Soxhlet flask, and repeat the extraction three times. Distil off the ether, dry the flask in the steam oven, cool in a desiccator and weigh. Dissolve out the fat from the flask by repeated extraction with petroleum ether, leaving any residue in the flask, dry the flask in the steam oven and weigh. By difference, find the weight of fat obtained and calculate the fat percentage.

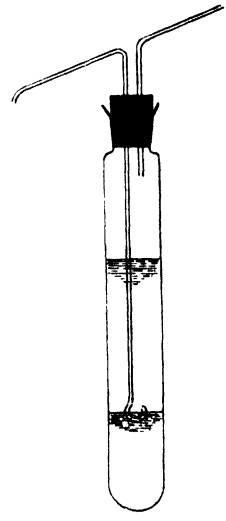


FIG. 14.—Werner Schmidt tube.

The Adams Paper Coil Method. This method will not be described in detail, although it possesses the advantage that the extraction of the fat is automatically effected in the Soxhlet extractor, and also that a number of determinations can be carried on simultaneously. It has not the general application to milk products of the two previous methods. The chief feature of the method is the distribution of a known weight of milk (about 5 gm.) on a strip of fat-free filter paper from which the fat is extracted when dry.

Volumetric Methods. The Gerber. The two most favoured methods are the Gerber and the Babcock. Both methods have the advantages of speed and suitability for routine work. The former is more generally employed in this country and will be described; the latter is widely used in America, the country of its origin. The Gerber method has been much improved since its introduction in 1892, particularly by better calibration of the graduated tubes employed for the reading of the volume of fat, and also by the avoidance of errors due to impurities in the chemicals used in the test. There is considerable choice in the kind of stopper used with the tubes, some of which are depicted. It is strongly recommended that all glassware needed for the test bear the stamp of the National Physical Laboratory, showing that it has been tested and that the errors do not exceed a low value. Cheap, uncertified apparatus is

often useless. The chemicals used are, (1) sulphuric acid of specific gravity not greater than 1·825[1] or less than 1·820[1] at 60° F., this is acid containing 90-91 per cent. of the strong acid; (2) amyl alcohol of specific gravity not greater than 0·816[1] or less than 0·814[1] at 60° F. The amyl alcohol should, however, always be tested when a fresh supply is purchased, by comparing the results obtained in several fat determinations with the results obtained by using the Gottlieb method with the same milks. Alternatively, a new consign-

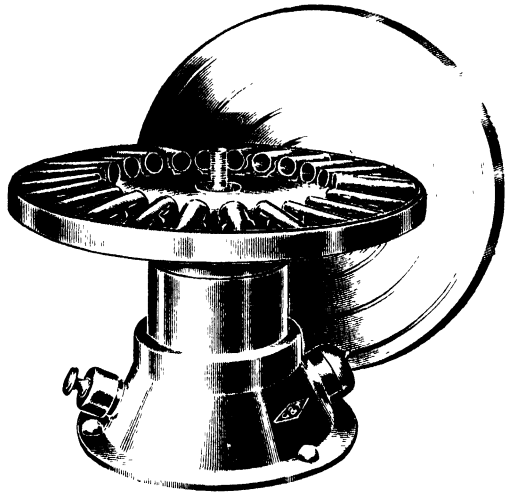


FIG. 15.—Gerber's centrifuge.

By courtesy of Messrs. Griffin and Tatlock, Ltd.

ment can be checked by means of a sample which has been so tested. A centrifuge is an essential part of the apparatus, and of these there are many types, ranging from small hand-driven machines for two and more tests, to machines for many tests, fitted with electric motors. The machine should be capable of 1000 to 1200 revolutions per minute.

If the precautions regarding equipment are observed, the method is capable of a degree of accuracy of within + or − 0·05 per cent. of fat.

Determine the percentage of fat in a sample of milk as follows, and compare the readings obtained in duplicate tubes.

Place the testing tube (butyrometer tube) upright in the stand with the open end upwards, and measure into it, either by an acid pipette or by an automatic measure, 10 ml. of the sulphuric acid,

[1] The density of the acid at 20° C. (68° F.) is 1·815. The density of the amyl alcohol at 20° C. is 0·810-0·812.

avoiding contact of the acid with the neck of the tube. Now measure with the milk pipette 11 ml. of the sample of milk which has been well shaken, and deliver this slowly on to the surface of the acid. The milk must not be mixed with the acid at this stage.

FIG. 16.—Types of stopper for Gerber's butyrometer.

FIG. 17.—Gerber's butyrometer showing reading of the fatty layer.

Now measure into the tube by pipette or automatic measure 1 ml. of the amyl alcohol. Fit the rubber stopper into the tube, thoroughly shake, and mix the contents by inverting the tube two or three times, and immediately place the tube in the centrifuge. (If only one test is being carried out, a second tube filled with water should be placed opposite in the centrifuge to preserve the balance of the machine.) Centrifuge at 1000-1200 r.p.m. for three to four minutes, slow down the speed gradually by holding a duster at the centre of the disc, lightly at first and then increasing the pressure until the machine stops. Remove the tube and adjust the bottom of the fat layer by careful manipulation of the stopper until it coincides with one of the long lines (which represent whole numbers) on the calibrated scale of the tube. With the fat layer in this position, read the point on the scale where the bottom of the meniscus of this layer coincides. The percentage of fat is the difference between the readings of the bottom and top levels (see Fig. 17). With a little practice the readings can be made with almost a glance. Immediately after use the tubes must be emptied and thoroughly washed.

The underlying principle of the method is the solution in the acid, of the casein and most of the other non-fatty solids of milk (the white deposit at the bottom of the tube after centrifuging is calcium sulphate and strikingly illustrates the amount of calcium in milk), and the amyl alcohol assists in the separation of the fat. Where many tests are carried out with one operation of the centrifuge, or the temperature of the room is low, it may be necessary to place the tubes in a water bath at 65° C., but with a warm room and the number of tests in the machine restricted to not more than eight, an expeditious worker may find little difference between readings as the tubes are taken from the centrifuge and readings after immersion in a bath. On no account, however, should the tubes be allowed to get cold.

Use of Figures for Specific Gravity at 60° F. and for Density at 20° C. for the Calculation of Percentage of Solids Not Fat

(*a*) **Lactometer.** The relationship between specific gravity at 60° F. and proportions of fat and non-fatty solids, can be expressed by the formula :

Solids not fat percentage

$$= \frac{\text{Lactometer degrees at } 60° \text{ F.}}{4} + 0{\cdot}2 \text{ Fat\%} + 0{\cdot}14.$$

Richmond's slide rule is based on this formula, but gives readings of total solids from which the percentage of fat must be deducted to ascertain the percentage of solids not fat. In using Richmond's slide rule the arrow on the slide is placed against the fat percentage and the percentage of total solids is read against the corrected specific gravity. With careful working the results for normal bulk milk are usually close approximations to those obtained by gravimetric determination. It should, however, be realised that the formula is a general statement (in mathematical form), and may not apply rigidly to a specific case.

(*b*) **Density Hydrometer.** As density at 20° C. and specific gravity at 60° F. are not the same thing the Richmond formula has been recalculated to the following :

Solids not fat percentage

$$= \frac{\text{Density hydrometer reading at 20° C.}}{4} + 0{\cdot}21 \text{ Fat\%} + 0{\cdot}66.$$

Since the introduction of the density hydrometer, work has been published[1] from which it appears that when the method is carried out as prescribed, the modified formula gives percentages of solids not fat approximately 0·2 lower than the gravimetric method.

From the specific gravity at 60° F. and/or density at 20° C. and the fat percentage calculate the percentage of solids not fat by the appropriate formula.

Determination of Proteins

Proteins are not the only nitrogenous substances present in milk, and, in consequence, the proportion of total proteins is only approximately determined from the nitrogen content. This determination, however, is easily carried out and finds favour in routine practice.

For research purposes and for the examination of milk samples from cows suffering from sub clinical mastitis the partition of the nitrogenous constituents of milk assumes importance. The methods given below by which the partition is effected are substantially those worked out by Rowland.

Determination of Total Nitrogen. Determine the percentage of nitrogen in a sample of milk by the Kjeldahl method (p. 28), but use 25 ml. of strong sulphuric acid for the digestion, 25 ml. of 0·1N. acid to receive the ammonia, and 5 ml. of milk (ascertain weight).

The result may be expressed as per cent. of total proteins (approximately) by multiplying the nitrogen percentage by 6·38.

Determination of Non-protein Nitrogen. The proteins are precipitated and separated from other nitrogenous substances by filtration.

Pipette 10 ml. of milk into a weighed 50 ml. graduated flask and again weigh. Fill to the mark with a 15 per cent. solution of trichloracetic acid, mix well and allow the precipitate to settle. Filter through a dry 9 cm. good quality filter paper into a dry

[1] Boden and Campbell, 1942. *J. Dairy Res.*, **13**, 45.

receptacle. Transfer 20 ml. of the filtrate (equivalent to 4 ml. milk) to a Kjeldahl digestion flask and after digestion determine the nitrogen content as above, using 10 ml. of 0·02N. acid in the receiver.

Determination of Non-casein Nitrogen. The basis of this method is the precipitation of the casein by bringing the medium in which it is dispersed to a certain intensity of acidity, which frees it completely from the bases with which it is combined in milk as caseinogen, and at which the casein is least soluble. This is termed the isoelectric point ; for casein this is *p*H 4·6. A low *p*H value, i.e. an increase in acidity, would cause some of the casein to dissolve. The casein is then freed from other nitrogenous substances by filtration.

Pipette 10 ml. milk into a weighed 100 ml. flask and again weigh. Add 70 to 80 ml. of distilled water at 40° C., 1 ml. of a 10 per cent. solution of acetic acid, mix and allow to stand 10 minutes. Add 1 ml. of N. sodium acetate solution (13·6 per cent.) to bring the *p*H to 4·6, mix again, cool to 20° C. and make up to the mark with distilled water. Mix well and allow the precipitate to settle. Filter through a dry 11 cm. good quality filter paper into a dry receptacle. Transfer 20 ml. of the filtrate (equivalent to 2 ml. milk) to a Kjeldahl digestion flask and determine the nitrogen content as above using 15 ml. of 0·02N. acid in the receiver. In calculating the result, correction should be made for the volume occupied by the precipitate of casein and fat which is normally 0·5 ml. per 10 ml. of milk, i.e. the 100 ml. flask will contain only 99·5 ml. of solution and the 20 ml. taken for the distillation is consequently equivalent to slightly more than 2 ml. of the original milk so that the result should be multiplied by 0·995.

Casein Nitrogen and Casein. By subtraction of the percentage of non-casein nitrogen from that of total nitrogen, the percentage of casein nitrogen is obtained. This figure multiplied by 6·38 represents the percentage of casein.

Further methods for the quantitative separation of the proteins of milk do not appear to warrant inclusion as students' exercises, but it should be realised that the proteins not determined by the methods described, viz. albumin, globulin, and a class described as proteose-peptone constitute respectively

about 9, 3½ and 4 per cent. of the nitrogenous constituents of milk.

Determination of Total Proteins by Formaldehyde Titration. The proteins of milk are neutral to phenolphthalein, but upon addition of formaldehyde they become acid, see p. 115.

Advantage can be taken of the principle of this reaction for the determination of proteins by direct means, but since the formulae of proteins are unknown, equations cannot form the basis for the necessary calculations. In milk, the determination of proteins by " formaldehyde titration " has in the past led to unnecessary and unscientific methods of stating the results. The process of determination is expeditiously carried out by Pyne's method.

To 10 ml. of milk add 0·4 ml. of saturated potassium oxalate and 0·5 ml. of phenolphthalein. Allow to stand for two minutes, neutralise with 0·1N. sodium hydroxide and read the burette. Add 2 ml. of neutral 40 per cent. formaldehyde and continue the titration. From this calculate the percentage of protein.

1 ml. of 0·1N. NaOH = 0·174 gm. protein.

N.B.—Formaldehyde is always acid and the 2 ml. added must either be neutralised before its addition, or 2 ml. separately titrated and a deduction made from the final reading to correct for it.

The acidity produced by reaction of formaldehyde with different proteins will, of course, vary with the amino acids of the proteins. In milk, however, the ratio between the casein and albumin is sufficiently constant to allow the total proteins to be calculated with reasonable accuracy.

Determination of Lactose

Two direct methods for this are given, both of which are rapid in practice. An indirect method is also described.

(*a*) **By Polarimeter.** Before the lactose in milk can be determined by means of the polarimeter, it is necessary to remove completely the fats and proteins, which interfere by making the solution too opaque for reading, and by polarising to the left. These substances are usually removed by means of solutions of mercury compounds. Acid mercuric nitrate is suitable

for the purpose, and the addition of a solution of this substance to milk causes the proteins to be precipitated as insoluble mercury compounds. After this treatment, filtration will give a clear solution of the lactose suitable for polarisation.

The usual form of polarimeter has, in addition to the scale graduated in angular degrees, a second scale called the International sugar scale. This sugar scale is so graduated that it provides direct readings in percentages for sugars, if the appropriate weight (called the normal weight) of the sugar is dissolved in 100 ml. and the polarisation made in a tube 200 mm. in length.

The normal weight of lactose for use with this scale is 32·9 gm. This weight of pure lactose when dissolved in 100 ml.

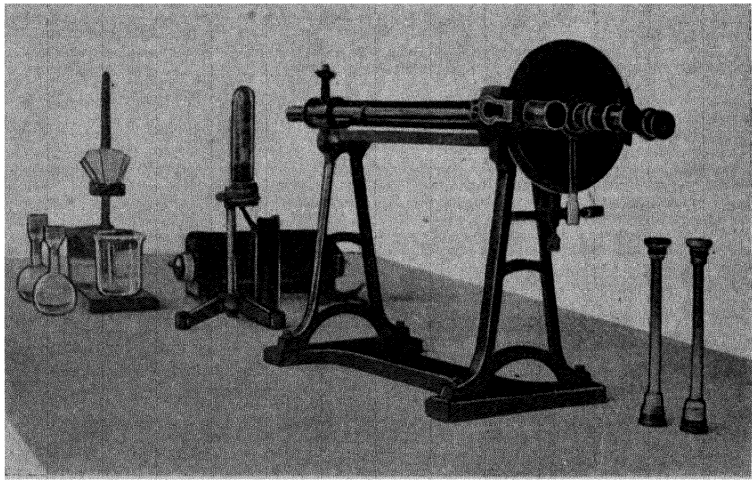


FIG. 18.—Polarimeter with its equipment.

and the solution polarised in a 200 mm. tube, will give a rotation of 100° on the sugar scale. From this it follows that if 32·9 gm. of a sample of lactose of 50 per cent. purity was made into a solution of 100 ml., and polarised in a 200 mm. tube, it would give a reading of 50°. In other words,

100 per cent. sugar = 100° rotation.
50 ,, ,, = 50° ,,

In the case of milk, the normal weight (32·9 gm.) is too small a quantity for convenient accurate determination, and it is usual to use twice this amount, viz. 65·8 gm. and divide the reading by 2 to obtain the percentage of lactose.

This figure, however, may be corrected for the volume which is occupied by the protein precipitate, since the lactose of the milk is not dissolved in a 100 ml. but in 100 ml. minus the volume of the precipitate. This can often be ignored, as the actual volume of the precipitate is much less than the apparent volume, owing to its colloidal nature. For accurate work the double dilution method of correction may be employed. In this method, one polarisation is made on the clarified solution which has been made up to 100 ml. as usual, and a second polarisation is made on another sample of the same weight which has been clarified and diluted to 200 ml. The underlying principle of this process lies in the fact that, while the amount of lactose and the volume of the precipitate are constant, the volume in which the lactose is dissolved varies. The polarisations of two solutions containing the same weight of the same substance are inversely proportional to their volumes, and the true rotation is the product of the two readings, divided by their difference.

Weigh 65·8 gm. of milk into a 100 ml. flask, add 3 ml. of acid mercuric nitrate, shake well and make up to the mark. Filter through a dry filter paper.

Fill a 200 mm. polarising tube with distilled water, screw on the cap of the tube and place in the trough of the polarimeter. Adjust the instrument until the field is uniformly illuminated and read the scale to ascertain zero. Empty the tube and rinse out several times with some of the filtrate containing the lactose, then fill with the solution and screw on the cap. Place the tube in the polarimeter, again adjust until the field is evenly illuminated and read the scale. The difference between the two readings divided by 2 = per cent. lactose.

N.B.—It is presumed that the student will have learned the underlying principles of the polarimeter, and that detailed instructions for using the instrument of the particular maker will be given by the teacher.

(*b*) **Iodometric Method.** Aldose sugars, such as lactose, glucose and maltose, are oxidised by iodine in the presence of sodium hydroxide, the iodine being removed from the solution as sodium iodide. This is the basis of the following method for the determination of lactose in milk.

Pipette 10 ml. (ascertain the weight) of the milk into a 100 ml. graduated flask. Dilute with 50 ml. of water, add 10 ml. of Mayer's reagent to precipitate the proteins and fat, and then add 2 ml. of normal sulphuric acid. Make up to the mark with water, shake well and filter. Pipette 25 ml. of the filtrate into a 250 ml. conical flask, neutralise with sodium hydroxide, add 20 ml. of 0·1N. iodine solution and 30 ml. of 0·1N. sodium hydroxide. Stopper and leave for twenty minutes. Add 4 ml. of normal sulphuric acid, and titrate the excess iodine with 0·1N. sodium thiosulphate solution, adding starch solution near the end of the titration.

A blank determination using 10 ml. of water in place of the milk should also be carried out at the same time. Subtract the number of ml. of thiosulphate used, from that required in the blank determination, to give the number of ml. of 0·1N. iodine used in oxidising the lactose.

1 ml. of 0·1N. iodine = 0·01705 gm. lactose.

Allowance has to be made for the volume occupied by the protein and fat precipitate, and for the fact that 25 ml. of the filtrate only was used ; the calculation of the percentage of lactose thus becomes :

$$\text{ml. of iodine used} \times 0{\cdot}0682 \times \left[\frac{100 - (0{\cdot}3 + F \times 1{\cdot}11)}{\text{wt. of milk taken}}\right]$$

where F is the weight of fat in the weight of milk taken.

(*c*) **An Indirect Method for the Determination of Lactose in Milk.** A method which is capable of approximate accuracy, and which has distinct educational value, is based upon the following.

The osmotic pressure of genuine fresh normal milk is very constant. Chlorides and lactose are mainly responsible for the osmotic pressure, any decrease in the amount of one being compensated by an increase in the other by an amount which would give the same osmotic pressure. E.g. 1 gm. of sodium chloride is equal in this respect to 11·9 gm. of lactose.

The osmotic pressure of milk due to chlorides and lactose is equal to that of a 7 per cent. solution of lactose. For every 51 mgm. of chlorine per 100 gm. of milk (0·051 per cent.) the lactose content is lowered 1 per cent. from a hypothetical maximum of 7.

For instance, in a sample containing 125 mgm. Cl per 100 gm.

$$\text{Lactose per cent.} = 7 - \frac{125}{51} = 4{\cdot}5.$$

Since chlorides are volatilised when milk is ignited, it is preferable to oxidise the organic matter of the milk by a wet process.

Pipette 10 ml. of milk into a small conical flask (250 ml.), and add 10 ml. of 0·05N. silver nitrate, 2 ml. of saturated potassium permanganate and 10 ml. of concentrated nitric acid. Boil the contents until clear, and add 100 ml. of water. Titrate the excess of silver nitrate with 0·05N. potassium thiocyanate, using 1 ml. of iron alum solution as indicator ; the end point being marked by the formation of ferric thiocyanate which colours the solution red. Since 10 ml. of 0·05N. silver nitrate is capable of precipitating 0·01775 gm. of chlorine, from the excess calculate the approximate percentage of lactose.

THE DETECTION OF ADDED WATER IN MILK BY DETERMINATION OF FREEZING-POINT

Although considerable attention was paid to the freezing-point of milk by early workers, its significance as a criterion of the genuine nature of milk was not fully appreciated in this country until 1921, when its determination became an official test in America. Although genuine milk is extremely variable in composition, its osmotic pressure in the fresh state is remarkably constant, as might perhaps be expected from the fact that it is manufactured from the blood of a species. It is well known that osmotic pressure and freezing-point are related, but the latter is more easily determined. It must be emphasised, however, that the constancy of the freezing-point of milk is only true of fresh milk, and that markedly acid milk is

valueless for the test to be described. The freezing-point of normal fresh milk lies between $-0{\cdot}53°$ and $-0{\cdot}56°$ C. The addition of water to milk causes the freezing-point to be nearer 0° C. in proportion to the amount added. Although perhaps complete unanimity on the point has not been reached, it may be stated that samples of milk with freezing-point nearer to 0° C. than $-0{\cdot}53°$ C. can be regarded as adulterated by the addition of water. The analyst is thus enabled to distinguish with almost complete certainty between milk which is genuine but possibly of low non-fatty solids content, and milk which has been watered. The certainty, of course, increases with the extent of such watering.

The apparatus for the determination (the cryoscope) is now standardised in the form recommended for administrative purposes as the Hortvet cryoscope. As the aim is not to ascertain the true freezing-point, since this would involve many experimental difficulties, and be impracticable where numbers of samples have to be examined, the Hortvet method may be classed as conventional and demands rigid adherence to the standardised technique in order that the apparent freezing-point observed under such conditions can be described as $-°$ C. (Hortvet), or by omitting the minus sign, as freezing-point depression (Hortvet).

The essential parts of the apparatus are (1) an outer chamber (which is insulated from the air by a vacuum jacket) in which ether vaporised by a current of dry air is the cooling agent; (2) a middle chamber containing a small quantity of alcohol, which acts as a heat-conducting medium between the milk and the freezing bath, so enabling the milk to be more rapidly cooled, and (3) an inner chamber for the milk which also carries an efficient stirrer and a very delicate and accurate thermometer for the measurement of the freezing-point. The scale of this thermometer covers a range of only 3° C. and is graduated in hundredths of a degree, but is capable with a good lens of being read to one thousandth of a degree. Each degree covers about four inches of the scale. Since so much

depends upon the accuracy of this scale, it is obvious that the calibration should be checked. In most cases it is desirable to ensure that this has been done before the thermometer is purchased by obtaining with it a certificate issued as a result of test by the National Physical Laboratory.

The accuracy of the thermometer can, however, be checked by observing the readings of its scale for the freezing-point of water and for solutions of sucrose of known strength. The following solutions are required for the purpose.

Gm. of pure (AR) sucrose per 100 ml. of solution at 20° C.	*Freezing-point depression (Hortvet)*
7·0	0·422° C.
8·0	0·487° C.
8·5	0·520° C.
9·0	0·553° C.

The commonly accepted formula for the calculation of the amount of water in samples of milk observed to have a freezing-point depression less than 0·53 is based upon the usual mixture law

$$W = \frac{T - T_1}{T} \times 100.$$

Elsdon and Stubbs,[1] however, consider that the following modification gives more accurate results

$$W = \frac{T - T_1}{T} \times (100 - T.S.)$$

Where W = Percentage of added water.
T = Freezing-point depression of genuine milk.
T_1 = Freezing-point depression of the sample.
$T.S.$ = Percentage of total milk solids in the sample.

In practice T could be the freezing-point depression of an "appeal to the cow" sample or the minimum freezing-point depression recognised for pure milk, viz. 0·53° C.

The Effect of Acidity on the Freezing-Point. It has been stated above that for the purpose of establishment of watering,

[1] 1936, *Analyst*, **61**, 383.

the test is invalidated if the milk has become markedly acid. A Scientific Advisory Committee[1] has reported that where the acidity (determined as on p. 124, calculated as lactic acid) exceeds 0·18 per cent. but does not exceed 0·78 per cent., a reliable indication of the original freezing-point depression is given by the formula

$$FPD = AFP - (LA - 0{\cdot}18) \times 0{\cdot}34.$$

Where FPD = Freezing-point depression of original milk.
AFP = Freezing-point depression of the acid milk.
LA = Percentage of Lactic Acid (Weight in Weight) in the acid milk.

Following the instructions given below, but under supervision, ascertain the freezing-point depression of (*a*) a sample of fresh whole milk and (*b*) the same sample after the addition of a known percentage of water. Calculate the percentage of added water in (*b*).

If time allows repeat the tests after the samples have developed appreciable acidity.

By means of a funnel, pour 400 ml. of ether into the outer chamber of the Hortvet cryoscope and connect to a suitable blower or a good filter pump, in the manner which allows a current of dry air to pass through the ether until the control thermometer in the ether chamber registers about −3° C. Shut off the air current and make up the volume of ether to 400 ml. (as shown by the gauge on the instrument) to replace that lost by evaporation. Place sufficient alcohol in the middle chamber to fill the space between it and the bottom of the inner chamber and pour into the inner chamber 30 to 35 ml. of the milk, or enough to submerge the bulb of the Hortvet thermometer when the cork, which carries the thermometer and the stirrer which fits round it, is fitted into position.

Lower the tube containing the milk into the middle or alcohol chamber, resume the passage of air through the ether and operate the stirrer by pulling it up and down at the rate of one stroke every one or two seconds until the temperature of the cooling bath reaches −3° C. and the mercury in the Hortvet thermometer has receded to −1·54° to −1·74° C. At this degree of supercooling "seed" the milk in the freezing tube with a fragment of dry ice by inserting the freezing starter (a non-corrodible metal rod supplied with the instrument),

[1] 1936, Dept. of Public Health for Scotland.

which has been kept in contact with ice, through the aperture in the cork provided for the purpose. Note the rapid rise in temperature which results almost immediately. Withdraw the freezing starter and operate the stirrer slowly and carefully while the mercury column reaches its highest point and remains stationary at the apparent freezing-point. Tap the upper end of the thermometer lightly but sharply with a cork mallet or rubber covered rod to ensure that the mercury thread is not sticking and with the aid of the lens read the thermometer to 0·001° C., being careful to keep the eye level with the top of the mercury column.

PRESERVATIVES AND ADDED COLOURING MATTERS

Although the addition of preservatives or colouring matters to milk is illegal, the student should be familiar with tests for the commoner substances occasionally added.

To nine separate samples of milk add preservatives in the following approximate proportions and apply the tests given below.

No. 1. 5 drops of hydrogen peroxide per 100 ml. of milk.
No. 2. 0·1 gm. of boric acid per 100 ml. of milk.
No. 3. 5 drops of formalin per 100 ml. of milk.
No. 4. 0·1 gm. benzoic acid per 100 ml. of milk.
No. 5. 0·1 gm. of salicylic acid per 100 ml. of milk.
No. 6. 0·01 gm. potassium nitrate per 100 ml. of milk.
No. 7. 0·1 gm. sodium bicarbonate per 100 ml. of milk.
No. 8. Sufficient cheese annatto to give a rich colour.
No. 9. 1 ml. of methyl orange per 100 ml. of milk.

Hydrogen Peroxide. Add to 10 ml. of the milk about an equal bulk of fresh milk (to ensure the presence of peroxidase) and a few drops of ortol solution or paraphenylene diamine solution. A red colour with the former, and a blue colour with the latter reagent, indicates the presence of peroxide.

Boric Acid or Borates. Evaporate about 2 ml. of the milk in a crucible and ignite to a white ash. When cool add one to two drops of a 0·01 per cent. solution of quinalizarin in 90 per cent. sulphuric acid. A blue colour develops if borate is present.

Formaldehyde. To about 5 ml. of milk and an equal bulk of water in a test tube, pour carefully down the side of the tube commercial strong sulphuric acid. A violet ring at the juncture of the liquids indicates formaldehyde.

Confirmatory Test. Add a few drops of Schiff's reagent to about 10 ml. of the milk. Development of a pink colour denotes formaldehyde.

Benzoic Acid. Test the milk with a few drops of ferric chloride solution. A buff colour indicates benzoic acid.

Salicylic Acid. Tested in the same way, a violet colour indicates salicylic acid.

Nitrates. To about 10 ml. of milk add 1 ml. of 10 per cent. acetic acid, filter into a test tube and pour carefully down the side of the tube a few drops of a solution of diphenylamine in concentrated sulphuric acid. A blue colour in the bottom layer or at the junction of the liquids indicates the presence of nitrate. (The presence of nitrates in milk may be indicative of the addition of water.)

Sodium Bicarbonate is not a preservative, but is sometimes added to milk. If the ash of 5 ml. of the milk contains soluble alkali requiring more than 0·3 ml. 0·1N. acid to neutralise it, sodium bicarbonate has been added.

Colouring Matters. *Azo Dyes.* The presence of an azo dye is indicated if the milk becomes pink on the addition of dilute acid.

Annatto. Make the milk alkaline with sodium carbonate, and soak in it a piece of cotton wool or filter paper. After some hours the wool or paper is turned brown if annatto is present, and will turn pink on the addition of stannous chloride solution.

SOUR MILK

The analysis of badly soured milk has sometimes to be undertaken by analysts in order to deduce the composition of the milk before souring occurred, but this is seldom the case in the dairying industry. Samples which are too sour to allow of the use of the lactometer, or of proper sampling for the Gerber method, may, however, be met with. The following procedure for samples which are easily poured is simple.

Measure 100 ml. of the curdled milk into a flask, add 5 ml. of diluted ammonia (strong ammonia diluted to 1 in 4), mix well and ascertain the lactometer reading. This reading will be lower than would be given by the original unsoured milk since the ammonia added has a lower density. Find the correction to be added, by ascertaining the depression of specific gravity which occurs when

5 ml. of the ammonia solution is added to 100 ml. of fresh milk on which a lactometer reading has been taken.

Determine the percentage of fat in the milk-ammonia liquid by the Gerber method, and increase the reading of the tubes by multiplying by 105/100 to correct for the dilution.

CREAM

It is important to note the legal definitions of this product of milk.

(1) Cream is " that portion of milk rich in milk fat which has been separated by skimming or otherwise and is intended for human consumption."

(2) " 'Cream' means that portion of natural milk rich in milk fat which has been separated by skimming or otherwise."

There is a clear legal distinction between cream and artificial cream, the latter article being defined in the Artificial Cream Act of 1929 as " an article of food resembling cream, and containing no ingredient which is not derived from milk ". Artificial cream is now a familiar product in many households, since it is cheaply and readily made with an inexpensive emulsifier or " cream gun". In the sale of this article the word " cream " must be immediately preceded by the word " artificial ". No legal standard of richness of cream exists, and as a consequence, samples of commercial cream show wide variation in composition, as instanced by the following examples: thin cream 29 per cent. of fat, thick cream 56 per cent. of fat. The addition of colouring matter, thickening substance or preservative, is, however, illegal.

Determination of Fat. Either the Gottlieb method (p. 129) or the Werner Schmidt method (p. 129), using 1 to 2 gm. of cream, and adding 8 ml. of water before digestion, can be used with excellent results. In routine practice, however, the Gerber method is generally used, either with special tubes for cream or with the butyrometer used for milk, but as the scale of the latter does not usually extend to readings of more than 8 per cent. fat, some method of known dilution of the cream must be employed before the test is made. Many methods of dilution and correction of the observed reading

have been proposed,[1] but they offer little advantage over the simple method described, and may consume nearly as much time as the accurate Gottlieb method. Dilute the sample of cream provided by well mixing 5 gm. of the cream with 45 gm. of separated milk or water. Determine the fat percentage by the Gerber method, and multiply the result by ten to obtain the fat percentage of the cream.

As the percentage of fat varies inversely with the specific gravity of the cream, a formula connecting the two can be deduced, which allows the percentage of fat in cream to be ascertained with approximate accuracy from the density of the cream.

Estimation of Fat Percentage from Specific Gravity. If the cream is thin the density can be ascertained by the lactometer, but with cream of moderate consistency it is almost as rapid to use the specific gravity bottle as to dilute and use the lactometer, and so avoid the possibility of errors due to calculation.

Ascertain the specific gravity of the cream previously tested, and note the agreement of the fat percentage determined and that given by the formula :

Fat percentage $= 32{\cdot}5 - (\text{lactometer degrees} \times 0{\cdot}91)$,

or $32{\cdot}5 - [(\text{sp. gr.} \times 1000) - 1000] \times 0{\cdot}91$,

or with rich cream of density below 1,

$$32{\cdot}5 + [(1 - \text{sp. gr.}) \times 1000] \times 0{\cdot}91.$$

Determination of Total Solids. Apart from fat, the constituents of genuine cream are present in the same relative proportions as in the milk from which it was prepared. The addition of water would disturb the usual ratio,

$$\text{Solids not fat} = \frac{\text{Water}}{10},$$

and the percentage of solids not fat determined would be lower than this figure. In the case of clotted cream and pasteurised cream the normal relationship between the constituents is disturbed by the evaporation of water.

Determine the percentage of total solids in the sample of cream used in the previous exercise, using about 5 gm. of cream. The

[1] British Standards Institution publication No. 696, part 2.

drying in the steam oven may take six hours. Subtract the fat percentage found, and note the agreement of the solids not fat percentage with that calculated from the formula.

SEPARATED MILK

As a check on the efficiency of the separator, the separated milk may be periodically tested for fat content. The Gerber method may be used for the purpose, and a " precision " butyrometer tube employed to allow of greater accuracy in the reading of the fat layer. The Gottlieb method is, however, much more reliable for this product.

Separated milk containing less than 8·7 per cent. of solids not fat is presumed by law to be watered, until the contrary is proved.

CONDENSED MILK AND DRIED MILK

The general agricultural student is perhaps little concerned with these products, but as the dairy " factory management " student may be, they are briefly discussed.

Condensed Milk. The sale of condensed milk is subject to The Condensed Milk Regulations of 1923, which prescribe conditions for correct description on the label regarding the grade of the milk and the number of pints of milk of that grade to which the contents of the receptacle are equivalent. The label must also state if the milk is sweetened or unsweetened. There are two grades of unsweetened condensed milk, viz. Condensed Full Cream and Condensed Skimmed. The same grading is adopted for sweetened condensed milk. Sweetened condensed milk is not sterilised, but preserved by approximately 40 per cent. of sucrose contained in it.

In the case of full cream milk, the statement regarding the number of pints of milk equivalent to the package sold, must refer to milk containing not less than 12·4 per cent. of milk solids (including not less than 3·6 per cent. of fat) ; the skimmed milk referred to must contain not less than 9 per cent. of milk solids other than fat. One pint of such whole milk (sp. gr.

1·0322) would contain 72·55 gm. of total milk solids and 21·06 gm. of fat, and one pint of such skimmed milk (sp. gr. 1·0355) would contain 52·84 gm. of milk solids other than fat. The statement of equivalent pints declared on the label of the receptacle can thus be checked by analysis of its contents.

Ascertain the weight in gm. of the tin of unsweetened full cream milk provided. Empty the contents into a beaker and again weigh the tin. Determine the percentage of total solids by the evaporation method, using 3 gm. of the sample, and determine the percentage of fat by the Gottlieb method on not more than 3 gm.

Calculate the equivalent pints on each basis and accept the lower figure.

$$\text{Equivalent pints} = \frac{\text{Total milk solids per cent.} \times \text{net weight in gm.}}{7255}$$

or

$$\text{Equivalent pints} = \frac{\text{Fat per cent.} \times \text{net weight in gm.}}{2106}.$$

For condensed skimmed milk the calculation is :

$$\text{Equivalent pints} = \frac{\text{Solids not fat per cent.} \times \text{net weight in gm.}}{5284}.$$

Dried Milk. The sale of dried milk is subject to The Dried Milk Regulations, 1923, and Amendment Regulations, 1927, which prescribe for correct labelling and description. The Regulations also apply to the dried milk contained in any powder or solid of which not less than 70 per cent. consists of dried milk. A statement of the equivalent pints of milk of the grade to which the dried milk purports to belong, is also required to be affixed to the container. The table given shows the statutory minimum limits for dried milks, and of the milks on which the statement of equivalent pints is to be based.

	Fat percentage of :	
	Milk Powder.	*Liquid Milk.*
Dried full cream milk - -	26	3·6
,, ¾ ,, ,, - -	20	2·7
,, ½ ,, ,, - -	14	1·8
,, ¼ ,, ,, - -	8	0·9
,, skimmed ,, - -	—	—

Dried skimmed milk is much used in mashes for poultry and in the rearing of calves.

Determination of Protein and Fat. Determine the nitrogen percentage and from it calculate the protein content using the factor 6·38 (p. 134).

For the fat determination the Werner Schmidt method is the best (p. 129), using 1 gm. of the sample and mixing with 9 ml. of water before digestion with the hydrochloric acid. Dry extraction of the fat in the Soxhlet extractor may give low results, since some fat may be embedded in particles of casein.

BUTTER

Butter is an emulsion, in which milk fat is the continuous phase, with milk serum (mainly water) dispersed in it. Some variation in the proportions of the various constituents, depending on the mode of preparation, is to be expected. The nature of the food of the cow may also affect the character of the fat and consistency of the butter, some feeding stuffs tending to produce soft, and other foods, hard butter. The vitamin A content of butter also is much influenced by the character and amount of green foods consumed. The substance which gives to butter its characteristic aroma is diacetyl, an oxidation product of acetyl methyl carbinol, which is produced by the organisms of the butter starter.

The extent of variation in composition commonly met is indicated below:

Water	11—14 per cent.
Fat	83—88 ,, ,,
Proteins	0·5—0·8 ,, ,,
Lactose and lactic acid	0·2—0·6 ,, ,,
Salt-free ash	0·1—0·2 ,, ,,
Salt	0—2 ,, ,,

The maximum percentage of water in butter allowed by law is 16.

THE ANALYSIS OF BUTTER (PROXIMATE)

Determination of Water. To obtain a representative sample for this determination, a large sample should be placed in a wide-mouthed bottle, warmed carefully until just melted, and then well shaken during cooling to obtain an even distribution of the water. For the determination, compare the results obtained in the two following methods :

(1) Accurately weigh about 3 gm. into a small porcelain dish and dry to constant weight in a steam oven.

(2) Weigh a small porcelain dish together with a glass stirring rod of suitable size, add 10 gm. to the weights, and counterpoise the dish with the butter. Heat the dish carefully over a small flame with constant stirring, until frothing and crackling cease and the casein is slightly brown.

In both cases calculate the percentage of water from the loss in weight.

Determination of Fat. Fat can conveniently be determined on the dried sample from the second method of moisture determination. Pour petroleum ether on to the contents of the dish and stir well with the rod. Allow to stand for five minutes for the curd and salt to settle, and then decant off the solvent. Repeat this extraction four times, and then place the dish on the water bath to drive off the last traces of solvent. Calculate the percentage of fat from the loss in weight. The residue is solids not fat and salt.

Determination of Ash. Ignite and weigh the residue from the previous determination. The loss in weight may be regarded as due to casein.

Determination of Salt. Two methods are described :

(1) Extract the salt from the residue from the fat determination with hot water, filter and wash six times to ensure complete extraction of the chloride, and determine its amount by titration with 0·1N. silver nitrate solution, using potassium chromate as indicator. The end point of the titration is marked by the formation of silver chromate (red).

$$1 \text{ ml. } 0{\cdot}1\text{N. } AgNO_3 = 0{\cdot}00585 \text{ gm. NaCl.}$$

(2) Extract the salt directly from the butter. For this process accurately weigh about 10 gm. of the butter into a small beaker, add about 20 ml. of boiling water and allow the butter to melt.

Transfer to a small separating funnel, shake thoroughly and allow the fat to separate. Carefully draw off the water layer into a graduated 250 ml. flask and repeat the extraction six times. Cool the washings to 20° C., make the volume up to 250 ml., well mix and pipette 50 ml. into an evaporating dish. Titrate with silver nitrate solution as above.

EXAMINATION OF BUTTER FAT AND COMPARISON WITH OTHER FATS

The main problem in butter analysis is to establish that the fat is wholly milk fat or an admixture with other fats or oils, as in margarine.

Although butter fat differs from all other fats by containing a much higher proportion of fatty acids of low molecular weight, notably of butyric acid, the determination of the actual proportion of a particular acid is difficult and impracticable as a general analytical method.

As a consequence, a number of empirical and conventional methods for the examination of fat have been proposed from time to time and subsequently improved. Of all these chemical methods, the most generally useful is that now known as the Reichert-Wollny-Polenske method, with which is usually associated a refinement due to Kirschner. The figures obtained by the method may be defined as follows :

The Reichert-Wollny (*R.W.*) is the number of ml. of 0·1N. alkali required to neutralise the volatile and soluble fatty acids obtained under specified conditions from 5 gm. of fat.

The Polenske Number (*P.*) is the number of ml. of 0·1N. alkali required to neutralise the volatile and insoluble fatty acids obtained under the same conditions.

The Kirschner Number (*K.*) is the number of ml. of 0·1N. alkali required to neutralise the volatile and soluble fatty acids obtained in the R.W. method which form soluble silver salts. Since silver butyrate is the only appreciably soluble salt of silver, it follows that the K. number gives a better indication of the proportion of butyric acid than does the R.W.

Preparation of the Fat. A sample of clarified fat is obtained by melting a portion of butter in a beaker at a temperature not exceeding 60° C. When the curd and water have separated and the upper layer is clear and bright, decant the fat through a filter and collect the fat in a dry beaker or wide-mouthed bottle. (A steam oven with the door open is convenient for melting the fat, and for keeping the fat molten during filtration.)

The Analytical Process. Strict adherence to the prescribed conditions is essential.[1] In particular, all apparatus must be of the dimensions shown, and blank determinations should be carried out with the reagents (omitting the fat).

Weigh 5 gm. of the molten fat into the flat-bottomed tube provided, and allow this tube to slide gently down the neck of the distilling flask into 20 gm. of glycerol previously placed in it. (It is much more convenient to weigh the fat in this way rather than in the flask. The glycerol acts as a cushion, and there is no likelihood of the tube breaking the flask.)

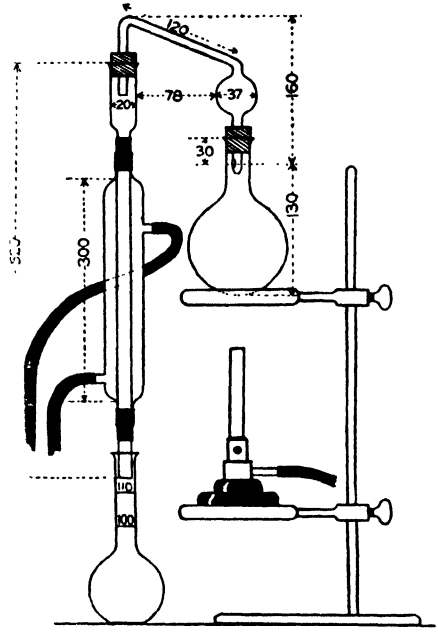


FIG. 19.—Polenske distillation apparatus for determination of volatile fatty acids.

Now add 2 ml. of 50 per cent. sodium hydroxide and heat over a naked flame with constant shaking until saponification has been effected. This is indicated by cessation of frothing, and the solution suddenly becoming clear. Allow the soap which has been formed to cool slightly, but not to set, and then add to it 93 ml. of hot distilled water which has been well boiled to expel carbon dioxide (this would otherwise render insensitive the indicator to be used later). Now add about 0·1 gm. of finely powdered pumice and then 50 ml. of dilute sulphuric acid (25 ml. concentrated sulphuric acid

[1] The method as described accords with the Society of Public Analysts report of 1936.

per litre, i.e. approx.N.). Note the precipitate of insoluble fatty acids which forms, and immediately connect the flask to the standard distilling apparatus under which is a graduated 110 ml. receiving flask. Heat the flask by a small flame until the insoluble fatty acids are melted, and then increase the flame so that 110 ml. of distillate are collected in nineteen to twenty-one minutes. Turn out the flame, remove the receiving flask and place under the condenser a small measuring cylinder. Remove the distilling flask and pour through the condenser tube 15 ml. of cold distilled water to wash the walls of the tube. This washing water is kept for the purpose of washing the insoluble volatile acids which are now filtered off from the 110 ml. of distillate, using a very small filter (9 cm.) and passing the whole of the distillate through it. The R.W. number is determined on 100 ml. of the filtrate by titration with 0·1N. barium hydroxide, using phenolphthalein as indicator. (0·1N. NaOH may be used if K. number is not required.) Since 100 ml. only of the 110 ml. collected from 5 gm. of fat have been titrated, the number of ml. of 0·1N. barium hydroxide used in the titration must be increased by 1/10th to give the R.W. number. The titrated liquid is reserved for the Kirschner determination.

The insoluble volatile acids which are on the filter, and perhaps on the walls of the condenser and original receiving flask, are now washed free from soluble acids with the 15 ml. of water, by pouring the water first into this flask and then on to the filter. Repeat the washing of condenser, cylinder, receiving flask and filter with two further portions of 15 ml. of water. This washing water is then rejected. Dissolve the insoluble acids by pouring through the condenser, and subsequently on to the filter, three successive quantities of 15 ml. of neutral alcohol, and collect the filtrate in the original receiving flask. The P. number is ascertained by titration of the complete filtrate with 0·1N. barium hydroxide (or NaOH), again using phenolphthalein as indicator. Since the whole of the insoluble volatile acids from 5 gm. of butter have been titrated, the titre gives the P. number directly.

To the titrated liquid from the R.W., add about 0·5 gm. of powdered silver sulphate, allow the mixture to stand for at least an hour with occasional shaking and then filter. (The object is to form the silver salts of the soluble volatile fatty acids, and then liberate and distil the acids from those salts which are soluble.) Place 100 ml. of the filtrate in the distilling flask of the standard apparatus, add 35 ml. of boiled water and 10 ml. of the dilute sulphuric acid

(as previously used), together with 0·1 gm. of powdered pumice, and again distil to obtain 110 ml. of distillate in twenty minutes. The K. number is obtained by titrating 100 ml. of this distillate with 0·1N. barium hydroxide as before. Since on two occasions 110 ml. have now been distilled and 100 ml. portions titrated, the titre has obviously to be corrected to give the K. number by multiplying the titration figure twice by 11/10, i.e. by 1·21. A further correction has also to be employed for the volume of 0·1N. alkali used in the R.W. determination, since the 100 ml. of liquid containing the acids from that determination were diluted by x ml. of 0·1N. alkali during titration, and a 100 ml. portion only of this was distilled for the K. number. Thus the full correction of the titration figure for the K. number becomes :

$$\text{K.} = \text{ml. of 0·1N. alkali used} \times 1{\cdot}21 \times (100 + x)/100.$$

Notes on the R.W., P. and K. Numbers. The R.W. number of genuine butter usually falls between 24 and 32. The P. number varies from 2·0 to 3·2 within this range, and rises with the R.W. number. Thus a high P. figure may be associated with a high R.W. figure without suggesting that adulteration has been practised, but the converse would indicate adulteration. There is, in fact, in the case of genuine butter an approximate proportionality between the three values, which in the case of two of them is expressed by Richmond as

$$\text{P.} = (\text{K.} - 14) \times 0{\cdot}26.$$

Adulteration with coconut or palm kernel oil is inferred if P. is higher than $(\text{K.} - 10) \times 0{\cdot}26$.

For most practical purposes the relationship between the average R.W. and P. numbers of genuine butter can be expressed as $\text{P.} = (\text{R.W.} - 10)/7$.

The maximum P. allowable would be $\text{P.} = (\text{R.W.} - 6)/7$.

Since the K. number is practically a measure of the butyric acid —the high proportion of which characterises butter fat—it is of great service for determining the proportion of butter fat in margarine. It is illegal for margarine to contain more than 10 per cent. of butter. The calculation becomes :

$$\text{Percentage butter fat} = \frac{\text{K.} - (0{\cdot}1\text{P.} + 0{\cdot}24)}{0{\cdot}244}.$$

The values given by some fats and oils used in margarine manufacture are given below. The values for butter made from the milk

of the goat are included on account of the high P. number of such butter.

	R.W. No.	*K. No.*	*P. No.*
Coconut oil	7	2	15·5-18·5
Palm kernel oil	5	1·1	9 -11
Sesame oil	1 -2	—	—
Beef fat	0·3- 0·5	—	—
Goat's butter	21 -27	17 -19	5 - 9
Cow's butter	24 -32	20 -26	2 - 3·2

The student is advised to repeat these determinations on a sample of clarified margarine fat.

Iodine Absorption Number

The glycerides of unsaturated fatty acids readily combine with the oxygen of the air or with halogens. The extent to which fats and oils can combine with halogens under certain standard and specified conditions has been ascertained for the majority of the various fats and oils, on samples of unquestioned purity. These recorded results, and the limits of variation noted, are of great value by affording means of comparison of doubtful samples of purported identity, and also by assisting in the identification of an unknown fat or oil. Thus the Iodine Number or Iodine Value of genuine butter fat ranges from 28 to 42. Corresponding numbers for some oils used in margarine manufacture and for some others of agricultural importance are given below.

Palm kernel oil	10 - 17
Coconut oil	7·5- 10
Cottonseed oil	103 -115
Linseed oil	170 -200
Cod liver oil	155 -173
Beef fat	36 - 42

Wijs Method for the Determination of Iodine Absorption Number. This number is the percentage of halogen, expressed as iodine, which is absorbed by the fat or oil under the conditions outlined.

Accurately weigh about 0·75 gm. of butter fat into the stoppered flask provided. Dissolve the fat in 10 ml. of carbon tetrachloride and then add 25 ml. of Wijs solution. (A solution of iodine monochloride in acetic acid.) Stopper the flask, well mix and allow the mixture to stand in the dark for half an hour. At the same time a similar flask containing the same quantities of reagents, but no fat, should also be placed in the dark. The purpose of this "blank experiment" is to ascertain, under the conditions of the determination, the weight of iodine equivalent to the halogens contained in 25 ml. of Wijs solution.

At the end of half an hour add to each of the two flasks 20 ml. of 10 per cent. potassium iodide solution and 100 ml. of distilled water. An amount of iodine equivalent to the halogens of the original solution will be liberated in the blank experiment, and a smaller quantity, due to partial absorption, will be liberated in the flask containing the fat.

$$ICl + KI = KCl + I_2.$$

Ascertain the weight of iodine in both cases by titration with 0·1N. sodium thiosulphate solution, using starch as indicator.

$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI.$$

From the equation it will be seen that 1 ml. of 0·1N. sodium thiosulphate = 0·0127 gm. iodine.

From the difference between the weight of iodine liberated in the two flasks, the weight of halogen as iodine, absorbed by the weight of fat taken, is found. Calculate the gm. of iodine absorbed by 100 gm. of the fat, i.e. the Iodine Number.

N.B.—If time permits a sample of margarine fat may also be examined.

Qualitative Examination for Adulterants

The elucidation of the nature of the adulterants in adulterated butter is seldom the concern of agricultural or dairy students, and in consequence, tests for specific oils are excluded from this book. There are many such tests, and also hydrogenated or "hardened" oils can often be detected in a mixture, by the trace of nickel which they may absorb from the catalyst employed in the hydrogenation process. A 1 per cent. solution of dimethyl glyoxime in alcohol gives a red precipitate with

nickel compounds, and allows one part in two millions to be easily detected.

The Phytosterol Acetate Test. Of more fundamental importance is the realisation that butter fat, in common with other fats of animal origin, contains small amounts of the sterol cholesterol, and never contains phytosterol, the sterol which is characteristic of vegetable oils and fats. These substances are unsaturated alcohols of high molecular weight, and are present in the unsaponifiable portion of fats and oils. Vegetable and animal oils and fats can thus be differentiated, and the presence of phytosterol in butter would be positive proof of adulteration with oils of the former class.

Any reliable method for the examination of sterols obtained from fats or oils must of necessity be tedious. The following method, however, presents little difficulty if the work is carried out with care.

Examine separately a sample of pure butter fat and a mixture of butter fat with coconut oil. Boil about 100 gm. of clarified fat with about 100 ml. of alcohol for a few minutes. Decant the hot alcohol into a 300 ml. conical flask, and again boil the fat with a further 100 ml. of alcohol, and decant into the flask. The hot alcohol dissolves the sterol, which is thus obtained free from the large bulk of fat. Distil off half of the alcohol and then add to the contents of the flask 10 ml. of 70 per cent. caustic potash, and saponify by heating on a sand bath under a reflux condenser. Cool, add 50 ml. of water and 100 ml. of alcohol, shake well, and add 20 ml. of digitonin solution (1 per cent. in alcohol) to precipitate the sterols as digitonin sterides. Allow to stand for twenty-four hours. Filter, wash with a small quantity of alcohol to remove soap, and dry the paper in the steam oven. Detach the steride from the paper and boil it with 3 ml. of acetic anhydride, and so obtain the acetates of the sterols. These may be distinguished by their melting-points, but in order to do so they must first be purified. Evaporate the solution to a pasty condition, dissolve out the sterol acetates in 10 ml. of hot alcohol, filter while hot through a small filter into a very small beaker. Evaporate about one half of the alcohol, and allow the acetates to crystallise out. The process of recrystallisation from hot alcohol must be carried out five times. Determine the melting-points of the crystals.

Phytosterol acetate melts at 127° C.
Cholesterol acetate melts at 113° to 115° C.

If the melting-point of the sterol acetates obtained from butter is 116° C. or over, the presence of vegetable oil is certain.

Physical Methods for the Examination of Butter

Physical methods for the examination of butter fat as criteria of purity have been much employed, but the value of these tests has diminished with the incorporation into margarine of mixtures of fats with physical constants not widely different from those of butter fat. Of these methods, the determination of the refractive index is the most important, and has the great advantage of being speedy. In laboratories equipped with a Zeiss Butyro-Refractometer, the dairy student should become acquainted with its mode of operation, which is extremely simple. The fundamental principles are (*a*) refractive index increases with increasing molecular weight of the combined acids, and (*b*) with increasing unsaturation. In a rancid fat, the free fatty acids lower the normal refractive index. The refractive index is often given as the Zeiss number, i.e. the number read off directly on the scale of the instrument. At a temperature of 40° C. (the temperature generally used) butter fat gives readings varying from 40 to 45, coconut and palm kernel oils give lower readings, beef-fat and lard higher readings, and oleo-margarine readings of about 48.

A microscopic examination of butter may yield valuable information if a polarising microscope is available. Butter which has been melted (for the purpose of renovating) and margarine often show crystalline forms which are completely absent from butter which has not been melted.

Examine samples of pure and of renovated butter by placing small pieces on microscope slides and viewing with the one inch objective. With the selenite plate of the microscope in position between the slide and the lower nicol prism, pure butter will show a uniform colour, but renovated butter and margarine will give a field mottled with various colours. Examination should also be made without the use of the polariser, to ensure that the crystals are not those of salt or preservative.

NATURAL AROMA

The constituent of butter mainly responsible for its agreeable aroma and flavour is diacetyl, $CH_3.CO.CO.CH_3$, which may be present in amount varying from 0·2 to 2 parts per million. The source of the diacetyl is the starter used for ripening the cream, where it develops from the oxidation of acetyl methyl carbinol, $CH_3CO.CHOH.CH_3$, produced by the activity of citric acid fermenting bacteria normally present in the starter.

Diacetyl is volatile in steam and can be detected by the test given below, by causing it to react with hydroxylamine to form dimethyl glyoxime which forms a red salt of nickel if a soluble nickel salt is used for its detection.

$$CH_3.CO.CO.CH_3 + 2H_2N.OH = CH_3C(N.OH).C(N.OH).CH_3 + 2H_2O$$

Diacetyl + Hydroxylamine = Dimethylglyoxine.

Place about 2 oz. of butter without melting in the flask of an apparatus for steam distillation (as shown on p. 105). Collect about 50 ml. of the distillate in a small flask, add 3 ml. of a 20 per cent. solution of sodium acetate, 2 drops of a 20 per cent. solution of hydroxylamine hydrochloride and 2 drops of a 10 per cent. solution of nickel chloride. Fit the flask to an upright condenser and maintain a temperature of about 50° C. for about one hour. Transfer the solution to a small porcelain dish and evaporate to a small bulk. A red coloration or precipitate shows that diacetyl was present in the butter.

RANCIDITY

Butter may develop " off flavours " and other defects which seriously reduce its commercial value. Among the chief causes of such defects are the following :

Hydrolytic Rancidity. One of the chief causes of rancidity is the hydrolysis of the fat by fat-splitting enzymes, which, however, should be rendered inert by pasteurisation. The speed of hydrolysis is influenced by the initial acidity of the butter. Another type of hydrolysis is that in which trimethylamine is produced from lecithin, and is responsible for a fishy flavour.

Place about 50 ml. of cream in a 200 ml. conical flask, add 0·1 ml. of formalin and 1 gm. of pancreatin powder. Plug the flask with cotton wool and place in an incubator at 38° C. until the next laboratory period. Note the characteristic rancid smell due to the liberation of fatty acids.

Oxidative Rancidity (Tallowiness). This defect is mainly due to oxidation of the oleic acid radical. Butter fat does not absorb oxygen as soon as it is in contact with it, but passes through a period in which there is little absorption. This period is called the induction period, and the longer its duration the greater the keeping quality of the fat.

The test given below to foretell the keeping qualities of a fat by indicating its ease of oxidation was suggested by W. L. Davies.

Emulsify 1 ml. of butter fat and 1 ml. of methylene blue solution (0·25 per cent.) in 10 ml. of separated milk and incubate at 37°-40° C. until the blue colour is bleached. After bleaching, vigorously shake the contents of the tube and note the depth of blue which develops.

A fat not easily oxidised, i.e. of good keeping quality, should produce only a pale blue.

Chemical Test for Oxidised Fat (Kreis). Place about 10 ml. of melted butter fat from an aged sample, and 10 ml. from a fresh sample in two separate test tubes, add 10 ml. of concentrated hydrochloric acid and 10 ml. of a 0·1 per cent. solution of phloroglucinol in ether. Close the tube with a rubber stopper and shake vigorously for half a minute. Note the colour produced in the acid layer when the tubes are allowed to stand. A strong red colour is produced by largely oxidised fats.

Acceleration of Rancidity by Metals. The rate of development of rancidity defects in butter is enormously accelerated by the presence of minute traces of metallic salts, especially of copper and iron. These salts act as catalysts, and so small a proportion as one part per million may cause an appreciable shortening of the " induction period ", and so be responsible for rapid oxidation.

To each of two test tubes add one drop of lactic acid and about 10 ml. of melted fat and shake. Place a piece of bright copper wire

in one of the tubes and heat both tubes in a boiling water bath for two hours and then remove the wire. Examine from time to time for signs of bleaching or discoloration and for tallowiness. By comparison of the tubes, observe the effect of the copper.

The detection of minute amounts of copper in dairy products is of importance, since commercial milk normally passes over a cooler, the tinning of which may become defective. It may in such cases dissolve sufficient copper from the exposed parts to catalyse chemical changes in milk, and be responsible for the production of undesirable flavours.

Test the butter fat from the last exercise for copper. Pour the melted fat into a small evaporating dish and ignite to completely remove the organic matter. Allow the dish to cool and then add dilute hydrochloric acid and boil. Now add ammonia until neutral, and filter off all insoluble material (including any ferric hydroxide which would interfere with the test). To the solution add about one-tenth of its volume of an aqueous solution (0·1 per cent.) of sodium diethyldithiocarbamate. If copper is present, a golden brown colour will be produced. The test is extremely delicate.

CHEESE

Cheese is the ripened curd produced by coagulating skimmed milk, whole milk or cream with lactic acid or rennet. No legal standard for the product exists in this country, but in legal definition " it is the substance usually known as cheese, containing no fat otherwise than from milk." In cheese-making, the action of the rennet added to the milk is well known to be influenced by temperature, acidity, and the presence of soluble calcium salts.

Coagulation of Milk by Rennet.

The Influence of Temperature. From the same sample of fresh milk measure 200 ml. into each of three beakers. Stand one beaker in cold water and warm the others to 40° C. and 55° C. respectively, and maintain at these temperatures for not less than one hour. As quickly as possible stir in 1 ml. of diluted rennet extract (1 part in 10), and note the time needed for coagulation.

The Influence of Reaction. Into each of five beakers measure

90 ml. of milk and add, while stirring quickly, the following reagents:

Beaker No. 1. Add 10 ml. of water.
Beaker No. 2. Add 10 ml. of 5 per cent. sodium carbonate.
Beaker No. 3. Add 10 ml. of 3 per cent. lactic acid.
Beaker No. 4. Add 10 ml. of 0·5 per cent. lactic acid.
Beaker No. 5. Add 10 ml. of 1·5 per cent. acid sodium phosphate.

Place the beakers in a bowl of water at 40° C., allow them to attain this temperature, and add while stirring 1 ml. of diluted rennet extract. Note the time needed for coagulation and the character of the curd.

The Influence of Soluble Calcium Salts. Into three beakers measure 100 ml. of fresh separated milk.

Add to each 0·5 ml. of saturated potassium oxalate (to precipitate soluble calcium salts), place the beakers in a bowl of water at 40° C. and maintain them at this temperature. Treat the beakers as under.

Beaker No. 1. No calcium salts.
Beaker No. 2. Add 2 ml. of 5 per cent. calcium chloride solution (by pouring the milk into it and well mixing).
Beaker No. 3. Add 20 ml. of clear lime water and mix.

Add to each beaker while stirring 1 ml. of diluted rennet extract. Note the time of coagulation and character of the curd.

RENNET

Exceedingly small amounts of rennet are able to coagulate large quantities of milk. The strength of rennet preparations is usually stated as the volume of milk that will be coagulated by one part of rennet in forty minutes. It is important to be able to ascertain the strength of such preparations, and for the purpose of the test the temperature chosen is not so high as the optimum for rennet action (40° C.), but a temperature nearer those employed in actual cheese-making, viz. 35° C.

Determination of Rennet Strength. Make up 5 ml. of rennet extract or 0·5 gm. of rennet powder to 100 ml. with water, i.e. it is diluted twenty times for liquid extracts or two hundred times for solid extracts. Add to 100 ml. of separated milk of acidity 20° which has been brought to a temperature of 35° C., 1 ml. of the diluted rennet, stirring during the addition. Note the exact time

at which the addition is made and stir gently until coagulation sets in, when again note the time.

Example. Suppose 1 ml. of diluted rennet extract ($=\frac{1}{20}$ ml. original rennet) coagulated 100 ml. of milk in eight minutes.

Therefore 1 ml. original rennet would coagulate (100×20) ml. $=2000$ ml. of milk in eight minutes.

In eight minutes 1 ml. coagulates 2000 ml. of milk.

$\therefore$ in forty minutes 1 ml. coagulates $(2000 \times 40)/8$ ml. of milk

$=10{,}000$ ml. of milk.

Strength therefore is 10,000

If the above quantities of rennet and milk are used, the strength of the original rennet can be calculated by simple proportion.

$$\text{Strength} = \frac{80{,}000}{\text{Time in minutes}} \text{ for liquid extracts,}$$

or

$$\text{Strength} = \frac{800{,}000}{\text{Time in minutes}} \text{ for solid extracts.}$$

N.B.—In commercial practice, milk powder is used for standardising rennet.

QUANTITATIVE ANALYSIS OF CHEESE

A quantitative determination of the products of ripening of cheese curd is seldom made, and in the majority of cases the analysis of cheese is made to ascertain its freedom from adulteration. Such adulteration sometimes takes the form of "filling," by which is meant the addition of foreign fats to skimmed milk cheese, which may afterwards masquerade as whole milk cheese. Where this is suspected, the fat should be extracted and examined by the methods detailed under Butter on p. 153. It is illegal to sell such cheese without a qualifying description.

Sampling. Hard cheese is conveniently obtained sufficiently finely divided by rubbing on a bread grater. Soft cheese is lightly pounded in a mortar.

Determination of Water. Dry in the steam oven for six hours (in a weighed dish) 2 to 3 gm. of cheese prepared as above and then weigh. Put back in the oven for a further period and reweigh.

QUALITATIVE EXAMINATION OF CHEESE.

During ripening the complex insoluble nitrogenous compounds of the curd gradually become converted into simpler and more soluble compounds.

N.B.—The peptones and amino acids are difficult of direct detection, and do not appear in the following scheme of examination.

Prepare a water extract of hard cheese by grinding about 50 gm. of cheese in a mortar with clean coarse sand. Transfer to a flask containing about 200 ml. of water at 50° C., maintain the temperature for about half an hour and shake vigorously from time to time. Decant on to a filter paper of very open texture and retain both filter and filtrate.

The Insoluble Material.

Digest with about 200 ml. of 5 per cent. salt solution at 50° C. for half an hour and filter through an open texture filter paper. To the filtrate add 10 per cent. acetic acid until coagulation occurs. The coagulum is termed **Brine-Soluble Proteins.**

The Soluble Material.

To about 100 ml. add 5 ml. of 1% hydrochloric acid, warm to 50° C. until a precipitate settles and filter.

Precipitate

Dissolve in dilute NaOH and apply the biuret test for proteins (p. 77). The precipitate is **Paranuclein.**

Filtrate.

Exactly neutralise with dilute NaOH, then add 10 per cent. acetic acid and boil. Filter while hot.

Precipitate. (if any) is **Heat-Coagulable Protein.**

Filtrate.

Cool to 70° C. and saturate with zinc sulphate. Filter off the precipitate, dissolve in a little water, and apply the biuret test. Note the colour, which is characteristic of **Proteoses.**

Place about 50 ml. in a 200 ml. flask and make this into a cream with magnesia (MgO). Boil and test the vapours with red litmus paper for **Ammonia.**

Concentrate about 50 ml. to 5 ml. in a porcelain dish on a water bath. When cold, pour into 5 ml. of Uffelmann's reagent in a test tube. A yellow or greenish yellow colour develops in the presence of **Lactic Acid.**

Test a portion for **Lactose** by the methylamine test (p. 118).

Cheese takes a long time to dry, but the process is facilitated if the dish is tilted so that the fat runs to one end. Calculate the percentage of water in the cheese.

Determination of Ash. Ignite the residue from the previous exercise at dull red heat. Cool and weigh. Calculate the percentage of ash.

Determination of Salt. Extract the ash with hot water and filter. Titrate the solution with 0·1N. silver nitrate. Calculate the percentage of salt.

Determination of Fat. Ascertain the percentage of fat by the Werner Schmidt method described on p. 129, using one to two gm. of cheese.

Determination of Total Nitrogen. Determine the percentage of nitrogen by the Kjeldahl method and multiply the result by 6·38 to obtain an approximate figure for proteins. (It should be realised that owing to changes during ripening the nitrogen is not present wholly as protein.)

DAIRY DETERGENTS

In the interest of cleanliness and hygiene these materials are used wherever milk is handled. They possess, in more or less degree, properties of wetting the dirt or solidified milk constituents it is desired to remove, and of facilitating its disintegration and dispersion in such manner that it can easily be washed off leaving no film. It is unlikely that a single chemical compound will have all the desired properties and a mixture of substances may be required to produce the wanted effect. The substances mainly employed are sodium salts of weak acids, e.g. carbonate, phosphate and silicate, or sodium hydroxide.

QUALITATIVE EXAMINATION OF DAIRY DETERGENTS.

Stir about 5 gm. of the powdered detergent with three successive quantities of 20 ml. of alcohol, pouring the liquid through a filter.

<table>
<tr><td>Filtrate.
Add a few drops of phenolphthalein. A pink colour indicates the presence of NaOH (Free alkali).</td><td colspan="2">Residue.
Wash on the filter with cold water and test separate portions of the filtrate as under.</td></tr>
<tr><td></td><td>Sodium Carbonate.
Add a few drops of dilute HCl and note the evolution of CO_2, showing the presence of Carbonate.</td><td>Sodium Silicate and Sodium Phosphate.
Pour into excess of hydrochloric acid ; a white gelatinous precipitate indicates the presence of Silicate.

Evaporate this to dryness and heat gently to render the silica insoluble. Add dilute nitric acid, filter if necessary and add Ammonium Molybdate to the filtrate and warm. A yellow precipitate indicates the presence of Phosphate.</td></tr>
</table>

Solutions of detergents naturally undergo loss of strength in the course of use, either by their action upon the foreign matter it is desired to remove or by interaction with substances causing hardness in a water supply. Consequently it is of importance to check the strength of solutions used in washing tanks. The test usually made for this purpose is a measurement of the volume of standard acid solution required to neutralise a given volume of the solution to phenolphthalein, the result being expressed as caustic soda (NaOH) or " available alkalinity ". For washing tanks a figure of not less than 0·5 per cent. is usually considered satisfactory.

Pipette 10 ml. of the detergent solution provided into a conical flask, add 5 drops of phenolphthalein solution and titrate with 0·1N. hydrochloric acid until the pink colour disappears.

1 ml. of 0·1N. HCl = 0·004 gm. NaOH.

Calculate the percentage of available alkalinity of the solution.

CHAPTER VI

THE CHEMICAL EXAMINATION OF WATER

In his future career, the agricultural student is unlikely to undertake the complete analysis of water. Nevertheless, the nature and quality of water supplies is of such great importance in domestic life, in dairying operations and in horticulture, that the student should understand the significance of the results of water analysis, and be acquainted with the processes by which the results are obtained.

Collection of the Sample of Water. The size of the sample required will naturally depend upon the information required, but for the full examination to be described, a Winchester quart bottle is suitable. The bottle must be scrupulously clean and well washed before the sample is taken ; bottles which have contained ammonia should be avoided. The sample should be stored in a cool dark place. (If possible a sample from a domestic supply and from a pond or ditch should be examined.)

Preliminary Examination. If the sample contains much suspended matter, a convenient volume may be filtered through a weighed filter paper which is subsequently dried in the steam oven to constant weight. The colour of the water should be observed, and also the taste and smell. The taste may be compared with that of a good tap water ; the smell is best observed after gently warming a sample in a closed flask to about 40° C.

Order of Work. To economise time and for other considerations, it is well to commence the determinations in the following order. The results of all determinations are stated as parts per 100,000.

(1) Total dissolved solids.
(2) Free and Albuminoid Ammonia.
(3) Nitrates and Nitrites.
(4) Oxygen absorbed (organic matter).
(5) Dissolved Oxygen.
(6) Chlorides.
(7) Hardness, temporary and permanent.

Determination of Total Dissolved Solids. Evaporate 250 ml. of the filtered water in a weighed dish and dry in the steam oven to constant weight. After the weight has been recorded, heat the dish gently and note if appreciable blackening occurs.

Determination of Free Ammonia. The proportion of ammonia in ordinary water supplies is too small to allow of its determination by titration. It can, however, be determined after liberation and distillation, by matching the colour produced by its reaction with Nessler's reagent, with an equal

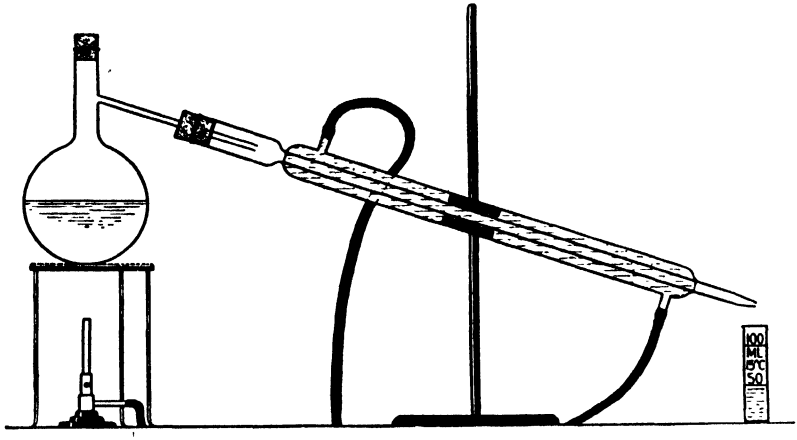


FIG. 20.—Distillation apparatus for water analysis.

volume of extremely dilute ammonium chloride solution of known strength, to which the same volume of reagent has been added. These colour standards do not keep. Where many determinations are made, the necessity of making fresh ones on each occasion is avoided by the use of coloured glasses of the same tints. The reagent is extremely sensitive, and the operation must be conducted in a room free from ammonia fumes.

Fit up a distillation apparatus as shown in Fig. 20, avoiding the use of rubber stoppers (these liberate ammonia). Place about one litre of tap water (not the water under examination) into the flask, add to it about a gm. of freshly ignited sodium carbonate and a small piece of pumice, and distil. Collect 50 ml. portions of the distillate in the graduated cylinders of colourless glass known as Nessler glasses, until no colour is produced on the addition of 2 ml.

of Nessler's reagent. The apparatus is now free from ammonia; and the water which subsequently distils must be collected in a clean flask for the preparation of the ammonia standards. Ordinary distilled water which has been exposed to laboratory air is quite unsuitable for the purpose.

When about 500 ml. of ammonia-free water has been collected, allow the distilling flask to cool a little and replace its contents with 500 ml. of the water to be examined. Add 1 gm. of ignited sodium carbonate and a small piece of pumice, attach to the condenser, and distil 50 ml. portions until these give no coloration with Nessler's reagent. The ammonia which is evolved has arisen from the decomposition of ammonium salts by the sodium carbonate; owing to its great solubility it is effectively collected in the condensed steam. Usually the first three 50 ml. portions of distillate contain all of the ammonia.

Prepare six colour standards by measuring 0·5, 1, 2, 3, 4 and 5 ml. respectively, of standard ammonium chloride solution (see Appendix) into six other Nessler glasses, make up to the 50 ml. mark with ammonia-free water, add 2 ml. of Nessler's reagent, and mix by rotating the glasses. Place the glasses on a white tile, and after standing for five minutes compare the colours of the glasses containing the distillate plus reagent with those of the standards. If the standards are not sufficiently deep in colour to obtain a match, further ones must be made containing more ammonium chloride. If, however, the colour is very strong, the distillate may be suitably diluted with ammonia-free water, and 50 ml. compared with the standards.

Example.

1st 50 ml. of distillate was matched by standard containing 3 ml. of NH_4Cl.

2nd 50 ml. of distillate was matched by standard containing 1 ml. of NH_4Cl.

3rd 50 ml. of distillate was matched by standard containing 0·5 ml. of NH_4Cl.

4th 50 ml. of distillate was matched by standard containing Nil.

Total ml. of standard ammonium chloride = 4·5.

The standard ammonium chloride solution contains 0·00001 gm. NH_3 per ml.

Hence 500 ml. of water contains 0·000045 gm. NH_3.

100,000 ml. of water contains 0·0090 gm. NH_3.

The water contains 0·009 parts of " Free Ammonia " per 100,000.

Determination of Albuminoid Ammonia. This operation is carried out on the water remaining in the distilling flask. Alkaline potassium permanganate solution, prepared as below, is added, and the distillation and collection of 50 ml. portions of distillate continued. The boiling alkaline permanganate solution liberates as ammonia the whole or a part of the nitrogen of nitrogenous substances present. The proportion so liberated depends upon the nature of the nitrogenous substance—it is termed " Albuminoid Ammonia."

Prepare the alkaline potassium permanganate by adding about 10 gm. of stick potassium hydroxide and 0·5 gm. potassium permanganate to about 40 ml. of water contained in a small flask. When dissolved, boil the liquid vigorously until it occupies about 20 ml., to ensure that it is free from ammonia. Allow the flask to cool a little, pour the contents into the large distilling flask and proceed with the determination as in the case of the Free Ammonia.

Determination of Nitrite and Nitrate Nitrogen. Nitrites and nitrates are reduced to ammonia by nascent hydrogen. The ammonia so produced can be volatilised and determined. A convenient, although rather slow, method of effecting reduction, is to utilise the hydrogen produced by the electrolytic action of a zinc-copper couple placed in the water.

Prepare a zinc-copper couple by rolling a piece of zinc foil about 3 × 2 inches in area into a cylinder of about half an inch diameter. Free this from grease by immersion in sodium hydroxide solution wash well with distilled water, and immerse in 3 per cent. copper sulphate solution until the zinc is covered with a firm deposit of copper. Place 100 ml. of the water under examination in a wide-mouthed stoppered bottle, place the metal couple in it and leave stoppered for about twelve hours or until the next laboratory period Transfer 50 ml. to the distilling flask, add ignited sodium carbonate and a fragment of pumice and proceed with the determination of ammonia as previously described. Note that the " Free Ammonia " is included in the determination and must be deducted.

Nitrites. Nitrites can be determined by comparing the colour produced on the addition of Greiss-Ilosvay reagent to a known volume of water, with the colours produced by the same reagent

when added to standard dilute solutions of sodium nitrite. The determination is rarely required, since nitrites should be absent from a good water supply.

Test the sample of water for nitrites, by adding 2 ml. of each of the two solutions from which the Greiss-Ilosvay reagent is made, to a test tube half full of the water. Allow to stand for about ten minutes. In the presence of nitrites a pink to red colour rapidly develops. Make a comparison test on the laboratory tap water.

Note. The reaction is extremely delicate and the tubes must be scrupulously clean.

Determination of Oxygen Absorbed (organic matter). A judgment of the amount of organic matter in water is formed from a measure of the oxygen required to oxidise it. In the process, a known volume of the water is acidified, and treated with a known excess of standard potassium permanganate solution. Under the acid conditions the permanganate readily gives up oxygen.

$$2KMnO_4 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 3H_2O + 5O.$$

The excess of permanganate remaining after the oxidation is measured by titration.

Measure into two large conical flasks labelled *A* and *B*, 250 ml. of the water and 250 ml. of distilled water respectively. To each add 10 ml. of diluted sulphuric acid (1 to 3), and 10 ml. of the standard potassium permanganate (0·396 gm. per litre), each ml. of which contains 0·0001 gm. of available oxygen. Stopper the flasks and place them in a water bath at 27° C. for four hours. At the expiration of this period, remove the flasks and add to each 10 ml. of potassium iodide solution (1 to 10). Iodine is liberated equivalent to the amount of permanganate present.

$$2KMnO_4 + 8H_2SO_4 + 10KI = 6K_2SO_4 + 2MnSO_4 + 8H_2O + 5I_2.$$

Titrate the iodine with sodium thiosulphate solution (2·5 gm. per litre), using starch as indicator, standardising this solution by titration of the iodine in the flask containing distilled water. From the amount of permanganate used for the oxidation of the organic matter, calculate the absorbed oxygen per 100,000 parts of water.

Example.

Flask containing distilled water required 15 ml. of thiosulphate.

Flask containing water examined required 10 ml. of thiosulphate.
Permanganate used by the water was equivalent to 5 ml. of thiosulphate.

Since 15 ml. thiosulphate = 0·001 gm. oxygen (10 ml. permanganate), oxygen absorbed by 250 ml. of water $= \frac{0{\cdot}001 \times 5}{15}$ = 0·00033 gm. or 0·132 part per 100,000.

Dissolved Oxygen. A corollary to the statement and exercise given under the previous heading is that the dissolved oxygen of a water supply will diminish on keeping if the water contains " active " organic matter. A valuable test of the purity of a water supply consists in measurements of its content of dissolved oxygen, before and after being allowed to stand for a period.

In the test described,[1] manganous hydroxide is produced by precipitation within a known volume of the water, the dissolved oxygen of which converts a part of it to manganic hydroxide. The precipitated hydroxide is then dissolved in hydrochloric acid and the higher manganese hydroxide, by reaction with potassium iodide, liberates free iodine which is measured by titration with a standard solution of sodium thiosulphate.

The essential reactions on which the test depends may be typified as under.

$$2Mn(OH)_2 + O + H_2O = 2Mn(OH)_3$$
$$2Mn(OH)_3 + 6HCl = 2MnCl_3 + 6H_2O$$
$$2MnCl_3 + 2KI = 2MnCl_2 + 2KCl + I_2$$
$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI.$$

Completely fill a glass stoppered bottle of about 300 ml. capacity with the water under examination, reserving a similar amount left at room temperature, for test after 5 days. By means of a pipette introduced well below the surface of the water, add 1 ml. of a 33 per cent. solution of manganese chloride and immediately after and in the same manner, 3 ml. of alkaline potassium iodide solution (see Appendix). Stopper the flask, taking care to exclude air bubbles, and mix the contents by inverting several times. After allowing about 15 minutes for the reaction between the oxygen and precipitated

[1] If the water contains nitrite this must first be oxidised to nitrate by means of permanganate and the excess of permanganate removed by potassium oxalate solution.

hydroxide to be complete, dissolve the latter by adding 3 ml. of concentrated hydrochloric acid. Transfer the contents of the bottle to a conical flask of about 500 ml. capacity and titrate the free iodine with 0·05N. sodium thiosulphate until the liquid is a pale yellow colour, add a few drops of starch solution and continue the titration until the blue colour of the starch iodide compound disappears. Ascertain the capacity of the bottle by filling with water and emptying the contents into a measuring cylinder and assume this to be the volume of water taken for the test, since correction for the volume occupied by the reagents would not appreciably affect the result.

Example.

From the above equations, 1 ml. of 0·05N. sodium thiosulphate = 0·0004 gm. oxygen.

Suppose volume of sodium thiosulphate used = 6·9 ml.
and volume of water taken = 300 ml.

Therefore Dissolved Oxygen in parts per 100,000

$$= (0{\cdot}0004 \times 6{\cdot}9 \times 100{,}000/300) = 0{\cdot}92$$

Since 1 ml. of oxygen at 0° C. and 760 mm. weighs 0·00144 gm., 1 litre of water contains 0·0092/0·00144 ml. = 6·4 ml. of dissolved oxygen.

Determination of Chlorides. Chlorides in water in appreciable quantity render the water unsuitable for many purposes, and may indicate pollution by sewage. It has, however, to be remembered that some public water supplies, which are bacteriologically very pure, have a high chloride content by reason of treatment with chlorine at the waterworks.

Chlorides in water can be determined by direct titration with silver nitrate solution, using potassium chromate as indicator.

Measure 100 ml. of the water into a porcelain basin, add a few drops of potassium chromate solution, and titrate cautiously, with constant stirring, with 0·01N. silver nitrate solution until a permanent faint red tint of silver chromate is observed.

1 ml. of 0·01N. silver nitrate = 0·000355 gm. chloride (Cl).

Determination of Hardness. A " hard " water is one which forms a lather with soap with difficulty. The degree or extent of hardness of waters is of great importance in many industries,

and to a large extent in horticulture, dairying and domestic life. Hardness is generally due to the presence in the water of calcium and magnesium salts, which combine with the fatty acids of the soap to form insoluble salts. Until sufficient soap is added to remove the calcium or magnesium salts, no lather is possible. Waters containing much sodium chloride will also cause loss of soap by its precipitation in an unaltered state. Hardness may be " temporary," i.e. removable by boiling, or " permanent," which cannot be so removed.

Two processes for the determination of hardness are in use. In Clark's method, a standard soap solution is added to 50 ml. of water until the point is reached at which a " permanent " lather forms. From the volume of soap solution used, the total hardness is expressed as parts of calcium carbonate per 100,000. The same process is applied after the water has been boiled, to ascertain the permanent hardness.

About half-fill three test tubes with :

(*a*) distilled water ;
(*b*) tap water ;
(*c*) tap water which has been boiled and cooled.

Add soap solution from a burette or dropping bottle, shaking after the addition of each drop until a lather is formed which lasts a few minutes. Note the number of drops required in each case.

In Hehner's method, advantage is taken of the fact that temporary hardness is caused by salts which are alkaline to methyl orange, i.e. the bicarbonates of calcium and magnesium. Consequently titration with standard acid gives a measure of the amounts of these compounds, which are expressed as their equivalent in parts of calcium carbonate per 100,000. Permanent hardness, which is due to the chlorides and sulphates of calcium and magnesium, is determined from the amount of sodium carbonate needed to precipitate these soluble salts as carbonates. The mode of expressing the result is as parts of calcium carbonate per 100,000.

The two methods do not give identical results ; there are

objections to both, but on the whole Hehner's method is to be preferred and is the only one described here.

Temporary Hardness. To 100 ml. of the water add a few drops of screened methyl orange and titrate with 0·1N. sulphuric acid.

1 ml. of 0·1N. acid = 0·005 gm. calcium carbonate.

Permanent Hardness. To 250 ml. of the water add 50 ml. of 0·1N. sodium carbonate solution, and boil for half an hour (the object of boiling is to remove salts causing temporary hardness). Filter into a 250 ml. graduated flask, wash the insoluble matter with hot water, cool and make up the volume. Shake well, pipette 50 ml. into a small conical flask, add a few drops of screened methyl orange and titrate with 0·1N. sulphuric acid. From the sodium carbonate used to precipitate the calcium salts, calculate the hardness as calcium carbonate.

1 ml. of 0·1N. Na_2CO_3 = 0·005 gm. calcium carbonate.

Example.

50 ml. of the treated and filtered liquid required 8·3 ml. of 0·1N. acid.

Hence 250 ml. would require 41·5 ml. As 50 ml. of 0·1N. solution were added, 8·5 ml. were used.

∴ parts calcium carbonate per 100,000

$$= \frac{8{\cdot}5 \times 0{\cdot}005 \times 100{,}000}{250} = 17.$$

THE INTERPRETATION OF THE RESULTS OF WATER ANALYSIS

It is generally admitted that no hard and fast standards can be fixed for drinking water, either from the results of chemical examination or from the bacteriological examination with which it is usually associated. The basis for judgment must largely be the geological formation from which the sample was drawn, and a general knowledge of the surroundings of the supply. If water from the same formation and district can be obtained, a comparison of the analytical figures may be made. Naturally, however, analysis cannot predict liability to pollution, or override in importance the application of common sense in regard

to the proximity of possible sources of pollution. In certain cases it might be desirable to examine the water for contamination by lead, copper, zinc, etc., or to ascertain the action of the water on metals by immersing strips of them in the water and subsequently testing the water.

N.B.—Analytical results are sometimes expressed as grains per gallon ; where this is considered advisable the conversion may be made by multiplying parts per 100,000 by 0·7. (One gallon = 70,000 grains.)

Total Dissolved Solids. A high content of dissolved solids is usually undesirable although not necessarily prejudicial to health. The scale left in boilers as the solid matter is deposited, is often troublesome and expensive to remove. Deep well waters usually contain 20 to 70 parts per 100,000.

Free Ammonia. In the decomposition of animal nitrogenous matter, ammonia is one of the first products to be formed. In the presence of bicarbonates of calcium and magnesium, the ammonia is readily oxidised to nitrites and nitrates. Its presence in quantity may consequently be indicative of recent pollution or absence of oxidation. The amount found in waters varies greatly, but suspicion may be aroused if it is more than 0·005 part per 100,000.

Albuminoid Ammonia. It has been explained that the ammonia liberated by boiling the water with alkaline permanganate, is derived from proteins and other nitrogenous substances. Generally, this figure should not exceed 0·008 part per 100,000 in the case of a domestic water supply.

Nitrites. In the process of nitrification, nitrites are quickly oxidised to nitrates, and consequently the presence of nitrites in any water but that obtained from a deep well indicates recent pollution. In the case of deep wells, the presence of nitrite and appreciable amount of nitrate points to past contamination.

Nitrite and Nitrate Nitrogen. The presence of more than 0·5 part per 100,000 arouses suspicion, although deep well waters may contain higher amounts.

Oxygen Absorbed in Four Hours. Waters which show greater

oxygen absorption than 0·1 part per 100,000 are usually classed as of doubtful purity.

Dissolved Oxygen. A normal water supply suitable for drinking and domestic use should contain 6 to 7 ml. per litre of dissolved oxygen and this amount should not diminish when the water is stored.

Chlorides. As in the case of the other figures, conclusions must not be too hastily drawn from a chlorine content higher than normal. The chlorine in water is usually present as sodium chloride, and may be derived from sea water. Land heavily manured with " potash salts " or with kainit will yield chloride to water percolating through it. As mentioned earlier, public water supplies may be chlorinated and have a high content of chloride (e.g. the Chelmsford supply contains 14 parts per 100,000). Where no such source of chloride can be traced, however, the presence of more than 4 parts per 100,000, which appears to be about normal, engenders suspicion of contamination by urine. Urine contains about 700 parts per 100,000.

Hardness. Although inconvenience is often caused by the hardness of domestic water supplies, it is unlikely that health is affected, except perhaps when a change of residence causes temporary indisposition. The degree and nature of the hardness is, of course, dependent upon the soil, but usually domestic supplies vary between 2 to 50 parts per 100,000. The suitability and treatment of hard water for making soap sprays is discussed under insecticides.

CHAPTER VII

FUNGICIDES AND INSECTICIDES

THE huge financial loss associated with the loss of crop due to insects and fungi detrimental to plant life, has led to the artificial control of these pests by the use of a large number of substances. These substances are usually applied in the form of fine dusts or mists or sprays.

It is beyond the scope of this book to discuss the time or manner in which these substances are applied, since much more than chemistry is involved; in fact, agriculture, pomology, entomology, mycology, physics and engineering have their parts to play in successful spraying practice.

It is, however, of importance that the student should be familiar with the nature and chemical properties of the substances in general use, and acquainted with the chemical methods employed to ascertain the purity, strength, etc., of these substances, and of the fitness for use as sprays of preparations made from them. From the financial point of view, also, the matter needs no stress, for it is obvious that failure to use insecticides and fungicides at the correct strength can only result in loss, which may well be much heavier than if spraying had not been resorted to. In the case of some proprietary materials, concerning which no details of composition are given, the best guarantee is the reputation of the manufacturer. Only materials well known to be standardised are dealt with in this book, but it is obvious that the list is capable of extension as standards become fixed for newer materials.

COPPER COMPOUNDS

Copper compounds are largely used as fungicides and are either purchased in concentrated compounded form, or are made up by the user by treating copper sulphate in the appropriate manner.

Copper Sulphate. Apart from its uses as fungicides, copper sulphate has long been used on the farm for " worming " sheep, and for spraying charlock. The commercial salt is marketed in a reasonably pure state, and a guarantee of 98 per cent. purity (98 per cent. $CuSO_4.5H_2O$) should accompany consignments to be used in the preparation of fungicides.

Carry out the following tests with copper sulphate solution.

(1) Ascertain the reaction to litmus paper of the solution.

(2) Place small pieces of zinc foil and some iron nails into portions of the solution, leave for a short time and note the chemical interaction. The above shows that iron and zinc vessels cannot be used for copper sulphate solutions.

(3) Pass hydrogen sulphide into some of the solution. A black precipitate indicates soluble copper.

(4) Add potassium ferrocyanide solution. A chocolate precipitate indicates soluble copper.

(5) Immerse a clean knife blade into copper sulphate solution. Note that copper is deposited from the solution.

Bordeaux Mixture. This popular and reliable fungicide is made by mixing copper sulphate and lime, or hydrated lime, in the presence of water. The relative quantities must be such that the whole of the copper is made insoluble ; soluble copper in the spray would be liable to cause injury to plants to which it was applied. The standard specification requires the copper (Cu) content of Bordeaux powders to be declared, with a statement that no water-soluble copper is present.

Prepare a little Bordeaux mixture by mixing the ingredients in the usual proportions. To 4 gm. of quicklime add a little water, allow the lime to slake and then make into a cream with 400 ml. of water. In another vessel dissolve 4 gm. of copper sulphate in 100 ml. of water, and when dissolved, add while stirring, to the milk of lime. Pour the mixture into a tall cylinder, and note that the precipitate gradually falls to the bottom. Rapid settling of the precipitate is undesirable. Withdraw some of the supernatant liquid, divide it into portions, and apply to it the tests for soluble copper described under copper sulphate.

In spite of much work, the chemistry of Bordeaux mixture

cannot be said to be fully worked out. The nature of the precipitate, which is generally regarded as a complex mixture of basic copper sulphates with excess of lime, is altered by varying the proportions of lime and copper sulphate, and more than one formula for making the fungicide is in use. Recent work suggests that only one basic sulphate, viz. $3Cu(OH)_2 CuSO_4$, is stable at ordinary temperatures, and that this reacts with excess of lime to form cupric hydroxide, which is probably stabilised by an adsorbed layer of calcium sulphate.

To show that the coloured compound differs from cupric hydroxide. To some copper sulphate solution contained in a test tube, add sodium hydroxide until alkaline to litmus. Heat the tube, and observe that the blue precipitate becomes black. Heat a little Bordeaux suspension, and note the absence of blackening.

Carry out the following tests.

(*a*) To a little Bordeaux mixture add an equal volume of 1 per cent. solution of soft soap. Note that soap cannot be mixed with Bordeaux mixture.

(*b*) Pour some freshly prepared Bordeaux suspension into two test tubes, and pass carbon dioxide through one for five minutes. Filter the contents of both tubes, and test the filtrates with potassium ferrocyanide solution.

Note that the action of carbon dioxide causes some copper to become soluble.

(*c*) Put some Bordeaux mixture aside and examine daily. Note that crystalline substances are deposited, and that Bordeaux mixture deteriorates on keeping.

Burgundy Mixture. This fungicide appears to equal Bordeaux mixture in efficiency, but to be rather more liable to cause injury to foliage on account of the soluble sodium salts.

Prepare a little Burgundy mixture by dissolving 5 gm. of washing soda in 50 ml. of water and pouring the solution, while stirring, into 350 ml. of water in which 4 gm. of copper sulphate have been dissolved. Observe if carbon dioxide is evolved by tilting the beaker so that any gas evolved may be poured into lime water. Pour into a tall cylinder, and note the characteristic precipitate ; when this has settled, withdraw some of the supernatant liquid and apply the

tests for soluble copper given under copper sulphate. Also test its reaction to litmus paper. The precipitate is a carbonate of copper containing an excess of base—a basic carbonate as, for example, $Cu_2(OH)_2CO_3$.

Determination of Alkalinity of Burgundy Powder. (The Prescribed Method.) The standard specification requires that such powders shall not show more than 2 per cent. alkalinity expressed as sodium carbonate (Na_2CO_3) when tested by the prescribed method.

Mix 10 gm. of the powder with about 10 ml. of water to break down all aggregates. Add about 100 ml. of water, stir until any apparent action ceases, and make up the volume to 250 ml. in a graduated flask. Shake well and filter. Pipette 50 ml. of the filtrate into a small conical flask and boil for two to three minutes, filter rapidly and wash any precipitate with a small quantity of hot, recently boiled water. Cool the filtrate, and titrate with 0·1N. sulphuric acid, using methyl orange as indicator. Calculate the alkalinity as percentage of sodium carbonate.

1 ml. of 0·1N. acid = 0·0053 gm. Na_2CO_3.

Cheshunt Compound. This fungicide is much used as a preventive of " damping off " of tomato seedlings.

The standard specification for this product is that it shall contain not less than 3·8 per cent. of copper when tested by the prescribed method.

Prepare a little Cheshunt compound by grinding together in a mortar 2 gm. of copper sulphate and 11 gm. of ammonium carbonate.

Dissolve a little of the compound in water and immerse a clean knife blade. Note that although copper is in solution as the cuprammonium ion, it is not deposited.

Determination of Copper in Bordeaux Powder, Burgundy Powder and Cheshunt Compound. (The Prescribed Method.) The method is based upon the measurement by titration with sodium thiosulphate, of the quantity of iodine liberated by the interaction of soluble cupric compounds with excess of potassium iodide. The reaction may be typified :

$$2CuSO_4 + 4KI = Cu_2I_2 + I_2 + 2K_2SO_4.$$

Weigh 5 gm. of the well mixed powder, and transfer to a beaker containing about 100 ml. of water. Stir well to break down aggregates, and add 5 ml. of glacial acetic acid or until effervescence ceases. Heat to boiling, add 5 ml. of 10 per cent. di-sodium phosphate solution to precipitate any ferric iron present, cool, transfer to a 250 ml. flask, dilute to the mark and shake. Filter and pipette 50 ml. of the filtrate into a small conical flask, add 4 gm. of potassium iodide, and titrate with 0·1N. sodium thiosulphate solution, using starch as indicator. Calculate the percentage of copper. From the equation

$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI$$

and the preceding one

1 ml. of 0·1N. sodium thiosulphate = 0·006357 gm. copper.

The thiosulphate solution should be standardised by titration of a solution containing a known weight (about 0·5 gm.) of highly purified copper sulphate, to which is added about 0·6 gm. of sodium carbonate crystals, 1 ml. of glacial acetic acid and 4 gm. of potassium iodide.

N.B.—The purpose of the sodium carbonate is to ensure that the thiosulphate solution is standardised under similar conditions to those of the copper determination.

LIME SULPHUR

The product of boiling a suspension in water of slaked lime with flowers of sulphur is a very popular fungicide. Great care has, however, to be exercised to ensure that the spray is used at the correct strength. The concentrated solution is usually purchased, as the reactions involved in its preparation are more under control in a factory than would be the case on farms. In spite of much work, the reactions which occur are not yet fully known, but it appears possible that polysulphides as high as CaS_7 may exist. It is suggested that the polysulphides of calcium possess the constitution : CaS. Sx. where x may be any number up to 6, a formula which distinguishes between the sulphur combined as monosulphide and the additional polysulphide sulphur. It is the loosely combined sulphur, i.e. that present in amount greater than needed for the formation of calcium monosulphide (CaS), which has fungicidal properties.

Prepare a specimen of "lime sulphur concentrate" by slaking 50 gm. of quicklime with a little water, and adding after slaking 100 ml. of water and 100 gm. of flowers of sulphur. Stir well until a thin paste is obtained, add a further 400 ml. of water and boil for half an hour. Allow the mixture to stand until undissolved material has settled out, and pour off the supernatant liquid through a linen filter.

Dilute some of the concentrate by mixing 10 ml. with 300 ml. of water, and carry out the following tests on the diluted wash.

(*a*) Leave a little of the liquid in contact with some copper turnings. Note that copper or brass vessels or sprayers should not be used with lime sulphur.

(*b*) Test the reaction to litmus paper.

(*c*) Add a solution of soft soap, and note that the mixture would be unsuitable as a spray.

(*d*) (1) Add dilute hydrochloric acid and note the smell of hydrogen sulphide and the precipitate of sulphur. The sulphur present in the hydrogen sulphide was combined in the spray as monosulphide; the sulphur which is precipitated was combined as polysulphides.

(2) To about 25 ml. add 10 ml. of ammoniacal zinc chloride solution, allow to stand for ten minutes and then filter. (The precipitate which falls contains the monosulphide and polysulphide sulphur.) Test the filtrate for thiosulphate (after neutralising the ammonia with a dilute solution of tartaric acid) by adding drop by drop a solution of iodine. Note that the iodine is decolorised.

(*e*) Arrange three measuring cylinders to contain :

(1) 100 ml. of water + 0·5 ml. of the 1 in 30 lime sulphur.

(2) 100 ml. of lime water + 0·5 ml. of the 1 in 30 lime sulphur.

(3) 100 ml. of dilute freshly prepared calcium thiosulphate solution. Allow the cylinders to stand, and note that sulphur is precipitated in (1), but not in (2) and (3).

At the end of twenty-four hours pass carbon dioxide through (2) and (3), and note that sulphur is not precipitated. In the case of (2) the gas must be passed sufficiently long to dissolve the precipitate of calcium carbonate which first forms.

The liquids in (2) and (3) have no fungicidal value ; the polysulphides of (2) have become converted into thiosulphate, e.g.

$$2CaS_5 + 7O_2 + 4Ca(OH)_2 = 4CaS_2O_3 + 2CaSO_3 + 4H_2O.$$

In actual spraying, sulphur is deposited as in (1), the polysulphides being oxidised or decomposed as illustrated by the equations :

(*a*) $2CaS_5 + 3O_2 \rightarrow 2CaS_2O_3 + 3S_2$.
(*b*) $CaS_5 + CO_2 + H_2O = CaCO_3 + H_2S + 2S_2$.

The fungicidal properties of lime sulphur solution are due to sulphur combined as polysulphides. It is the content of polysulphide sulphur which determines the value of the concentrated solution, thiosulphate and other sulphur compounds always present have little fungicidal power. The standard specification requires the purchased concentrate to be free from sludge, and to have a specific gravity of about 1·3. It shall not contain less than 18·5 per cent. weight in weight of polysulphide sulphur as determined by the prescribed method. This is equivalent to approximately 24 per cent. weight in volume.

Determination of Polysulphide. (The Standard Prescribed Method.) In this method the polysulphides react with ammoniacal zinc chloride to form unstable zinc polysulphides, e.g. ZnS_5, which are quickly decomposed into free sulphur and zinc sulphide, e.g. $ZnS_5 \rightarrow ZnS + 4S$.

The free sulphur reacts with sodium sulphite to form thiosulphate, which is titrated with standard iodine solution, after removal of the excess of sodium sulphite (which would also react with the iodine), by precipitation with strontium chloride. Prior to titration with iodine, the excess of ammonia is removed by neutralising with an acid, e.g. tartaric acid or acetic acid, which is too feebly ionised to allow of the formation of hydriodic acid, which would prevent the reaction going to completion (see also p. 192). Thiosulphate sulphur originally present is titrated at the same time, and must be separately determined and deducted in order to arrive at the polysulphide content.

Pipette 10 ml. of lime sulphur concentrate into a tared weighing bottle, weigh quickly, transfer by means of a water jet to a 250 ml. graduated flask, make up the volume and mix well. Pipette 25 ml. of the diluted spray into a 350 ml. conical flask containing 10 ml. ammoniacal zinc chloride solution, and 35 ml. of a freshly prepared 10 per cent. solution of crystalline sodium sulphite. Place the flask on a boiling water bath for forty-five minutes, shaking at intervals

of ten minutes, and washing down the sides with boiling water from a wash bottle. At the end of forty-five minutes remove the flask from the bath, add 30 ml. of 10 per cent. strontium chloride solution, allow to stand for five minutes and filter into a 250 ml. graduated flask. Wash the filter with hot water until about 200 ml. of filtrate and washings have been collected, cool, make up the volume and shake. Pipette 50 ml. into a small conical flask, add one drop of methyl red and then from a burette a 10 per cent. solution of tartaric acid, until the liquid is slightly acid. Titrate the thiosulphate with 0·05N. iodine, using starch as indicator and record the figure until it is required for the final calculation. The thiosulphate formed by the reaction between the liberated polysulphide sulphur of the spray with the sodium sulphite used as reagent, was contributed equally by both substances, as in the equation

$$S + Na_2SO_3 = Na_2S_2O_3$$

Under these conditions, therefore, 1 ml. of 0·05N. iodine = 0·0016 gm.[1] of polysulphide sulphur.

Calculate the percentage of polysulphide sulphur after deducting thiosulphate sulphur determined as below.

Determination of Thiosulphate Sulphur. Pipette 50 ml. of diluted spray into a 200 ml. graduated flask containing 50 ml. of water, add 20 ml. ammoniacal zinc chloride solution and make up the volume with water. Shake well and filter through a dry filter. Pipette 100 ml. of the filtrate into a small conical flask, add a few drops of methyl red and tartaric acid solution from a burette to exact neutrality. Titrate with 0·05N. iodine, using starch as indicator and record the titration figure.

Polysulphide Sulphur. Calculate the percentage of polysulphide sulphur as follows.

If X = the number of ml. used in the first titration and Y = the number of ml. used in the second titration, then, since the weight of sample taken in the second titration is 5 times that taken in the first titration, the true polysulphide titration figure is $X - Y/5$. Taking into account the weight (W) and dilutions of the original spray for the determinations, the final calculation becomes

[1] This value for 0·05N. iodine is, of course, one half of that required by the familar equation

$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI$$

which would be used if the percentage of thiosulphate sulphur is required.

Polysulphide sulphur per cent.

$$= (X - Y/5) \times 0{\cdot}0016 \times \frac{250}{25} \times \frac{250}{50} \times \frac{100}{W}.$$

COLLOIDAL SULPHUR

The colloidal state of matter (which for the purpose of this book may be considered as matter in an extremely fine state of subdivision, the particles usually being of less than microscopic size, and consequently of relatively large surface area) plays an important part in all life processes and in agriculture and industry.

The degree of success which follows the application of insecticides and fungicides is largely dependent upon the fineness of division of the material deposited upon the plant foliage.

In the case of sulphur, this has long been recognised, for not only does fineness of division allow of more frequent points of contact with the fungus it is desired to attack, but greater adherence to foliage is obtained.

Preparation of Colloidal Sulphur. To about 20 ml. of alcohol contained in a small flask add about 0·1 gm. of powdered sulphur. Place the flask on a boiling water bath until most of the sulphur has dissolved and decant the clear solution by drops into about 500 ml. of water. Note that the finely divided state of the precipitated sulphur allows a high degree of dispersion and that a stable suspension —a sol—results.

FORMALDEHYDE

Formaldehyde, or as it is often called commercially, Formalin, is much used as a seed dressing to kill fungus spores of the type of " bunt " and " smut ". It is also used as a fumigant, and for the partial sterilisation of soils. The standard specification requires it to contain not less than 36, and not more than 40 per cent. by weight in volume of formaldehyde.

Carry out the following tests with a weak solution of formaldehyde.

(1) Add ammoniacal silver nitrate solution and place the test

tube in a boiling water bath. Note the deposition of silver showing that formaldehyde is a reducing agent.

(2) Add a little Schiff's reagent and note that the solution becomes coloured.

Determination of Formaldehyde. Formaldehyde is oxidised to formic acid, and the latter measured by titration with standard alkali.

$$2HCHO + O_2 = 2HCOOH.$$

To 3 ml. of the sample add 50 ml. of hydrogen peroxide (10 vol.) and 50 ml. of N. sodium hydroxide solution, and warm until effervescence ceases. Titrate the excess of alkali with N. sulphuric acid, using phenolphthalein as indicator. At the same time make a blank determination (without formaldehyde). The difference between the two titrations represents the sodium hydroxide required to neutralise the formic acid produced by the oxidation of the formaldehyde.

1 ml. of N. sodium hydroxide = 0·030 gm. formaldehyde.

ORGANIC MERCURIALS

Organic compounds of mercury are much used for the purpose of destroying fungi on seeds and as a means of control of seed borne diseases. Many different compounds are used, nearly all of them being purchased as proprietary materials in which a relatively small amount of the active chemical is intimately mixed with a much larger quantity of inactive but adherent powder, the filler—so allowing it to be measured with sufficient accuracy on farms, in proportion to the amount of seed to be coated with the powder.

These organic compounds of mercury have proved more efficacious as fungicides and less damaging to seed than inorganic compounds of the element. They have largely displaced formaldehyde and copper sulphate for seed " dressing ".

Usually the proprietary powders incorporate a dyestuff which aids its recognition and also lessens the risk of " dressed " and " undressed " grain being confused.

About half fill a test tube with some grain which has been dressed with a proprietary organic mercurial preparatory to sowing, add 50 per cent. alcohol to nearly fill the tube, cork and allow to stand for

one hour with occasional shaking. Note if a dye colours the supernatant liquid.

Before tests can be applied for the detection and measurement of the mercury present, the organic compound must be oxidised and the mercury obtained in inorganic form. In the official method of analysis this is accomplished by heating the sample with concentrated sulphuric acid and hydrogen peroxide until all organic matter is destroyed. The solution is then diluted and the mercury precipitated and weighed as mercuric sulphide. For detecting the presence of mercury the simple method given below is satisfactory.

Place about 0·5 gm. of the fungicide in a small porcelain basin, cover with concentrated nitric acid and evaporate to complete dryness on a water bath. When cool add to the residue one or two drops of a freshly prepared saturated alcoholic solution of diphenyl-carbazone. A blue colour develops in the presence of mercury.

Place about 200 gm. of dressed grain in a small beaker, cover with dilute nitric acid and heat until nearly boiling. Decant the clear extract into a porcelain basin and evaporate to dryness. Cover the residue with concentrated nitric acid, again evaporate to dryness and add a drop of diphenyl-carbazone solution to the residue.

ARSENIC COMPOUNDS

Many insecticides rely upon arsenic for their killing qualities, and in view of the highly poisonous character of this element to plants and higher animals, it is important that the student shall become familiar with some of the properties of some of the principal arsenic compounds.

Arsenic forms two series of salts, arsenites and arsenates, which correspond to the oxides, arsenic trioxide, As_2O_3 (or arsenious oxide), and arsenic pentoxide As_2O_5. The former compounds are not used as insecticides as their solubility renders them unsuitable, very small quantities of water-soluble arsenic being injurious to young foliage.

Qualitative Tests with Arsenic Compounds.

Arsenious Oxide. (1) Place a little of the powder on blue litmus paper and moisten with water. Note that it is feebly acid.

(2) Shake a little arsenious oxide with water in a test tube and heat. Note that it is only slightly soluble in water. Divide the suspension into two portions, to one (*a*) add hydrochloric acid, and to the other (*b*) sodium hydroxide solution, and again heat. Note that the oxide now dissolves.

The reactions which occur are :

(*a*) $As_2O_3 + 6HCl = 2AsCl_3 + 3H_2O$.

(*b*) $As_2O_3 + 2NaOH = 2NaAsO_2 + H_2O$

sodium arsenite.

Pass hydrogen sulphide into the tube (*a*) and note the yellow precipitate of arsenious sulphide As_2S_3.

(3) Place a little arsenious oxide in a small porcelain dish, cover with strong nitric acid, and evaporate to dryness on a water bath in the fume cupboard. The nitric acid oxidises the As_2O_3 to As_2O_5. Moisten the residue with a little water, and test its reaction to litmus paper. Note that it is acid, due to the formation of arsenic acid H_3AsO_4.

Sodium Arsenite and Sodium Arsenate. Examine the laboratory specimens of sodium arsenite and sodium arsenate and carry out the following tests on aqueous solutions of them :

(*a*) Add magnesia mixture and shake vigorously.

(*b*) Add ammonium molybdate solution and warm.

(*c*) Add silver nitrate solution.

(*d*) Pass hydrogen sulphide.

(*e*) Make alkaline with sodium carbonate, and add iodine solution drop by drop.

(*f*) Pass sulphur dioxide until the solution smells strongly of the gas, then boil to expel the excess. Add sodium carbonate until alkaline, and then iodine solution drop by drop. Note (*a*) and (*b*) that no precipitate occurs with magnesia mixture and arsenites, and the similarity between arsenates and phosphates in their behaviour to these reagents. (*c*) In neutral solution arsenites give a yellow precipitate with silver nitrate, arsenates give a brown precipitate ; (*d*) arsenites give a yellow precipitate of arsenious sulphide ; (*e*) arsenites are oxidised by the iodine solution which is decolourised ; (*f*) arsenates are reduced by sulphur dioxide to arsenites.

Lead Arsenate. The principal arsenical insecticide is lead arsenate. Three forms of lead arsenate are known, all of which are insoluble in water : neutral lead arsenate $Pb_3(AsO_4)_2$, which is unstable, acid lead arsenate or di-plumbic arsenate

$PbHAsO_4$, and basic lead arsenate $Pb_5OH(AsO_4)_3$. The standard specification for the lead arsenate used in this country as insecticide implies that it shall be di-plumbic arsenate. This is a quick-acting poison, being hydrolysed as under :

$$5PbHAsO_4 + H_2O \rightleftarrows Pb_5OH(AsO_4)_3 + 2H_3AsO_4.$$

To a solution of lead nitrate add a solution of acid sodium arsenate ; note the precipitate of di-plumbic arsenate :

$$Pb(NO_3)_2 + Na_2HAsO_4 \rightleftarrows PbHAsO_4 + 2NaNO_3.$$

To a solution of lead acetate add a solution of acid sodium arsenate. Note the precipitate of neutral lead arsenate.

$$3Pb(C_2H_3O_2)_2 + Na_2HAsO_4 = Pb_3(AsO_4)_2[1] + 4NaC_2H_3O_2 + 2CH_3COOH.$$

Examine a commercial sample of lead arsenate. Dissolve a little by boiling with dilute nitric acid and cool ; test the solution as under :

(*a*) Add hydrochloric acid. A white precipitate which dissolves on boiling indicates lead.

(*b*) Add ammonia until alkaline, filter and add magnesia mixture to the filtrate. A white precipitate indicates arsenate.

Quantitative Examination of Lead Arsenate. The standard specification for lead arsenate is as follows :

Lead Arsenate Powder. It shall be a fine powder free from lumps and grit. It shall contain not less than 31 per cent. of arsenic, calculated as arsenic pentoxide (As_2O_5), not less than 63 per cent. of lead, calculated as PbO, and not more than 0·5 per cent. of water-soluble arsenic, expressed as As_2O_5. It shall conform to the limit test for acidity.

In the case of lead arsenate powders containing spreaders or wetting agents, the above limits may not apply, but the manufacturer should declare the percentages of arsenic, lead and water-soluble arsenic.

Lead Arsenate Paste. Shall be of uniform smooth consistency, free from lumps and hard particles, and shall readily rub down to a fine cream with water. It shall consist essentially of a

[1] $Pb_3(AsO_4)_2$ is unstable and probably hydrolyses as follows :

$$2Pb_3(AsO_4)_2 + H_2O = PB_5OH(AsO_4)_3 + PbHAsO_4.$$

mixture of di-plumbic arsenate ($PbHAsO_4$) and water. The paste shall contain not less than 14 per cent. of arsenic calculated as As_2O_5 and not less than 28·4 per cent. of lead calculated as PbO. It shall conform to the limit test for acidity, and shall not contain more than 0·5 per cent. of water-soluble arsenic expressed as As_2O_5 calculated on the dry basis. The manufacturer shall declare the percentage of arsenic expressed as As_2O_5 in the paste.

Determination of Total Arsenic. In this method, arsenates are first reduced to arsenites by sulphur dioxide, the excess of which is removed by boiling, prior to titration with iodine. The iodine oxidises arsenites as shown in the equation

$$As_2O_3 + 2I_2 + 2H_2O = As_2O_5 + 4HI.$$

Since the HI formed in the reaction is a reducing agent, it is necessary to remove it as it is formed, and sodium bicarbonate is added for this purpose.

Heat to boiling 1 gm. of lead arsenate powder, or 2 gm. of lead arsenate paste, for fifteen minutes with 25 ml. dilute sulphuric acid (1 vol. to 4 vols.). Add 25 ml. of water, filter and wash the filter until free from acid. Cool the filtrate and dilute to 250 ml. Reduce the arsenic acid in 50 ml. of the filtrate by passing sulphur dioxide through it until saturated. Complete the reduction by heating the liquid in a suitably stoppered vessel in a boiling water bath for fifteen minutes. Transfer the liquid to a flask, and boil off the excess of sulphur dioxide. Cool, add solid sodium bicarbonate until the solution is strongly alkaline, and titrate with 0·1N. iodine, using starch as indicator.

1 ml. 0·1N. iodine = 0·00575 gm. As_2O_5.

Determination of Water-soluble Arsenic. Mix 10 gm. of lead arsenate powder, or 20 gm. of lead arsenate paste, with 1 litre of recently boiled and cooled distilled water in a stoppered vessel of about 1½ litre capacity. Place in a warm place (about 30° C.), and shake at frequent intervals during four hours. Cool and filter through a dry paper. Reduce the arsenic in 50 ml. of the filtrate, and proceed as in the method for total arsenic, but titrate with 0·01N. iodine, using starch as indicator.

1 ml. 0·01N. iodine = 0·000575 gm. As_2O_5.

Paris Green. Paris green is a compound prepared by boiling a solution of copper acetate with arsenious oxide. It is an aceto-arsenite of copper to which the formula $3Cu(AsO_2)_2Cu(C_2H_3O_2)_2$ is ascribed. Although it was the first arsenical substance to be used as a spray, it is now little used for this purpose, it being difficult to keep suspended in the wash, and because of its proneness to cause injury to plants by high water-soluble arsenic content. It is, however, excellent material for poison baits for agricultural pests.

To a solution of sodium arsenite add a solution of copper acetate. Note the green precipitate of Paris Green.

NICOTINE. $C_{10}H_{14}N_2$

Nicotine is intensely poisonous, and because of this property its solutions make valuable insecticides, whether eaten, or absorbed through the skin, and further it is not injurious to foliage. Nicotine sulphate is also employed. The standard specification requires the manufacturer to declare the content of nicotine, and that it is substantially free from coal-tar bases.

Chemically, nicotine is an alkaloid, and it therefore gives the general reactions of this class of compounds.

Examine the laboratory specimens of nicotine and nicotine sulphate and make the following tests.

(1) Warm a solution of nicotine and note the smell of tobacco showing that nicotine is volatile.

(2) Repeat the test using a solution of nicotine sulphate, and after noting the smell add a few drops of sodium hydroxide and again note. The alkali liberates the nicotine from the combination with sulphuric acid.

(3) Test the reaction to litmus paper of a solution of nicotine, and note that it is alkaline.

(4) Add a solution of oxalic acid to a solution of nicotine and note the white crystalline nicotine oxalate which forms.

(5) Add a solution of silico-tungstic acid to a solution of nicotine previously acidified with dilute hydrochloric acid. Note the precipitate of nicotine silico-tungstate.

(6) Add Mayer's reagent to a solution of nicotine and note the precipitate. This reaction is given generally by alkaloids.

(7) To a solution of nicotine add one drop of formaldehyde and one drop of nitric acid. Note the rose colour which develops.

Determination of Nicotine. The nicotine is precipitated as nicotine-silico-tungstate which is converted into $SiO_2 12WO_3$ by ignition.

Introduce into a weighing bottle about 2 gm. of the sample and weigh. Transfer as much as possible to the distilling flask of a steam distillation apparatus, and reweigh the bottle. To the sample in the flask add 100 ml. of water and 10 ml. of sodium hydroxide solution (10 per cent.), and steam distil until the distillate is free from nicotine, i.e. is not alkaline to litmus paper. Dilute the distillate to one litre, shake well and pipette 50 ml. into a beaker. Add 10 ml. of 2N. hydrochloric acid and an excess (about 15 ml.) of silico-tungstic acid solution (12 per cent.). Stir until the precipitate crystallises and allow to stand for four hours. Filter and wash the precipitate with dilute hydrochloric acid (about 0·1 per cent.) until the washings are free from silico-tungstic acid, i.e. until a drop gives no blue colouration with stannous chloride paper, and then with a little water. Dry and ignite the precipitate. The residue $\times 0{\cdot}114$ = nicotine.

DICHLORO-DIPHENYL-TRICHLOROETHANE OR D.D.T. $(C_6H_4Cl)_2.CH.CCl_3$

This material is a powerful contact and stomach insecticide. Tests with D.D.T. and other closely related compounds indicate that in general their toxicity depends upon their ability to penetrate an organism and upon the ease of liberation of hydrogen chloride when in contact with sensitive internal tissues.

If D.D.T. is boiled with alcoholic potassium hydroxide one molecule of hydrogen chloride (HCl) is liberated from each molecule of D.D.T. This reaction forms the basis for the following method of determining D.D.T. in the concentrated material or in dusts containing D.D.T.

$$(C_6H_4Cl)_2.CH.CCl_3 + KOH$$
$$= (C_6H_4Cl)_2C{:}CCl_2 + KCl + H_2O.$$

Determination of D.D.T. Weigh about 0·5 gm. of D.D.T. concentrate or 5 gm. of a dust containing D.D.T. and transfer to a 500 ml. conical flask. Add 40 ml. of a normal solution of alcoholic potassium hydroxide and attach a condenser to the flask. Boil on a hot plate at 80° C. under the reflex condenser for 30 minutes. Allow to cool and pour through the condenser 50 ml. of water, 50 ml. of approximately 2N. nitric acid and another 50 ml. of water. Remove the flask and transfer the contents to a 250 ml. graduated flask. Add 20 ml. of 0·1N. silver nitrate with a pipette, shake vigorously, make up to the mark with water and thoroughly mix the contents. Filter through a dry filter paper and pipette 200 ml. of the filtrate into a conical flask. Add about 3 ml. of a saturated solution of iron alum as indicator and titrate the excess silver nitrate with 0·1N. potassium thiocyanate until the red colour of ferric thiocyanate remains permanent. Carry out a blank determination using the same amounts of D.D.T. and reagents, without heating, and titrate immediately. Calculate the percentage of D.D.T. present using the factor,

$$1 \text{ ml. of } 0{\cdot}1\text{N. } AgNO_3 = 0{\cdot}03545 \text{ gm. D.D.T.}$$

Example.

Let w = wt. in gm. of D.D.T. material taken.

x = ml. of 0·1N. KCNS used, i.e. the difference between the blank and back titrations.

$\therefore$ Percentage of D.D.T. $= x \times 250/200 \times 0{\cdot}03545 \times 100/w$.

HEXACHLOROCYCLOHEXANE OR 666 OR "GAMMEXANE" ($C_6H_6Cl_6$)

Hexachlorocyclohexane or benzenehexachloride is another powerful contact insecticide which acts in the same way as D.D.T. by releasing hydrogen chloride within the organism. The crude material is a mixture of at least four isomers, the alpha, beta, gamma and delta isomers. Of these the gamma isomer is highly toxic to insects while the others are relatively harmless. A commercial name for the gamma isomer is "Gammexane" and this is present to the extent of 10 to 12 per cent. in crude hexachlorocyclohexane. In practice, however, the mixture of isomers is used.

When boiled with alcoholic potash one molecule of hexachlorocyclohexane splits off 3 molecules of hydrogen chloride with the formation of a mixture of the isomers of trichlorobenzene.

$$C_6H_6Cl_6 + 3KOH = C_6H_3Cl_3 + 3HCl + 3H_2O.$$

Determination of Hexachlorocyclohexane (mixed isomers of 666). Weigh about 0·1 gm. of the concentrate or 4 gm. of a dust containing "Gammexane" and proceed with the determination exactly as described above for D.D.T. Calculate the percentage of mixed isomers using the factor,

1 ml. of 0·1N. $AgNO_3 = 0·0097$ gm. of 666.

SOFT SOAP

Soap solutions are much used in spraying, as they are very effective against soft bodied insects. They are also employed as spreaders when mixed with other sprays, to give more uniform spread and adherence of the spray to the foliage, and in making oil emulsions. The soaps most favoured are soft soaps, i.e. the potassium salts of the fatty acids of the fats or oils used in their manufacture. Whale oil and fish oils are very suitable for the manufacture of soft soaps used in spraying, as such soaps appear to be more penetrating and toxic to insects than those made from ordinary vegetable or animal oils. Resin soaps are effective against scale insects by reason of the clogging action of the resin, which has penetrated the trachea, and which settles out under the action of carbon dioxide.

Pass carbon dioxide through a strong solution of a resin soap and allow to stand until the end of the class. Note the resin deposited.

Soaps used for spraying should not contain excess of alkali, as this would injure foliage, and they should not be aged and carbonated.

The standard specification for soft soaps for spraying purposes is: "When tested by the prescribed methods, soft soaps for spraying purposes shall conform to the following requirements:

(1) It shall dissolve completely in distilled water to a clear solution.

(2) It shall contain not more than 1 per cent. of free caustic alkali calculated as potassium hydroxide (KOH), and not more than 3 per cent. of free alkali carbonate, calculated as potassium carbonate (K_2CO_3).

(3) Not less than 95 per cent. of the total alkali expressed as potash (K_2O) shall be potash (K_2O).

(4) The percentages of fatty and of resin acids shall each be declared."

Solution. Dissolve 2 gm. of the sample in 200 ml. of hot distilled water, cool the solution and note if it is clear.

Determination of Caustic Alkali and Alkali Carbonate. Potassium hydroxide and potassium carbonate can be separated by hot alcohol, in which the former is soluble and the latter insoluble.

Dissolve 20 gm. of the sample in about 75 ml. of neutral alcohol (95 per cent. alcohol which has been neutralised to phenolphthalein) by boiling under a reflux condenser. Filter the solution whilst hot, and wash the filter thoroughly with hot neutralised alcohol.

Caustic Alkali. Cool the filtrate, and titrate with N. sulphuric acid using phenolphthalein as indicator.

1 ml. of N. sulphuric acid = 0·056 gm. potassium hydroxide (KOH).

Alkali Carbonate. Wash the residues from the filter paper with hot distilled water; cool and titrate with N. sulphuric acid using methyl orange as indicator.

1 ml. of N. sulphuric acid = 0·069 gm. potassium carbonate (K_2CO_3).

Fatty and Resin Acids. The quantitative determination of these is tedious and is not included here as a student's exercise.

Examine one or two brands of soft soap for the presence of resin acids. To a strong solution of the soap add dilute sulphuric acid until acid, and heat in a water bath until the fatty acids separate. Transfer to a separating funnel and draw off the watery layer. Pour a little of the fatty acids into a test tube, add about one-third of the volume of acetic anhydride and warm. Cool the tube and pour cautiously down the side a mixture of equal parts of acetic acid and sulphuric acid. A purple colouration at the junction of the liquids indicates the presence of resin.

The amount of soap needed in relation to hardness of water. Of prime importance in spraying is the knowledge of the amount of soap needed to form a lather with the water used. Obviously

this will depend upon the quality of the soap and upon the hardness of the water. As far as the former is concerned, the " lathering power " of soaps with a standard water can be determined and compared, while for the latter, the hardness as determined on p. 176 may give guidance. From several points of view, however, it is better to determine the amount of the actual soap needed to form a lather with the water with which it will be used.

Dissolve 10 gm. of the soft soap in about 50 ml. of alcohol and dilute to one litre with distilled water. Shake well and fill a burette with the solution. Measure 50 ml. of the water into an eight-ounce bottle, and add the soap solution from the burette in quantities of about 1 ml. at a time, shaking vigorously after each addition until a muffled or softened sound is apparent when the bottle is shaken close to the ear. The solution should now be added more cautiously and the shaking continued after the addition of each 0·1 ml. until a point is reached when a lather is formed which persists for five minutes. From the burette reading deduce the amount of soap needed to soften the water. Using 50 ml. of water, each ml. of the soap solution corresponds to 0·2 lb. of soap per 100 gallons.

SPREADERS

These are substances which are added to sprays to improve their efficiency in various ways, (1) by increasing their wetting and spreading powers, i.e. to improve " coverage ", (2) to cause greater adherence to the leaf, i.e. to improve retention (the term " sticker " is now usually applied to such substances) and (3) by acting as emulsifiers or protective colloids and so increasing the stability of emulsions or suspensions. The properties of wetting and spreading are related to surface tension, for a full discussion of which books on physics should be consulted.

Wetting and Spreading. The importance of wetting and spreading agents is illustrated by the fact that when water falls as rain on to a cabbage leaf, the water collects as large droplets, which run off the leaf leaving the surface dry. In other words no stable liquid/solid interface is formed between water and the leaf. The addition of a spreader to a spray

promotes the formation of a liquid/solid interface between it and the sprayed surface and the area of the latter which remains covered with spray is thus a measure of the efficiency of the spreader.

Prepare a 0·1 per cent. solution of nicotine in water, pour about 100 ml. of the solution into each of four beakers, and treat as follows :

(1) Add 100 ml. of water.
(2) Add 100 ml. of a solution containing 2 gm. of lime casein.[1]
(3) Add 100 ml. of a 1 per cent. solution of soft soap.
(4) Add 100 ml. of a 0·8 per cent. solution of gelatine.

(*a*) " Paint " some of the nicotine solution from beaker (1) on a cabbage leaf and on a piece of glazed black paper or a piece of American cloth. Note that the liquid will not spread evenly over the leaf or other glazed surface but tends to run off or remain as separate droplets. Repeat the " painting " with the other nicotine solutions and note the improvement in wetting and spreading due to the addition of soap, casein or gelatine respectively.

(*b*) Dip a waxed card in each of the four solutions and observe on withdrawal the extent to which the liquid film retreats to form droplets.

The above experiments indicate that soap is more efficient as a wetter and spreader than either lime casein or gelatine.

Retention. If the surface tension of a spray is so reduced to allow perfect wetting of the foliage, substances added which increase the viscosity of the spray fluid also increase the amount of spray retained as a film on the leaves, i.e. increase retention. In the following experiment a chemically clean glass slide is used to represent a surface which can be perfectly wetted.

Prepare (*a*) 500 ml. of a solution of gelatine (about 0·5 per cent.) in distilled water by gently warming and leaving for 24 hours to attain maximum viscosity, and (*b*) 500 ml. of a 1 per cent. solution of soft soap.

Chemically clean a glass slide (3 in. by 1 in.) by immersing for some time in chromic acid solution, followed by washing successively with running tap water and finally with distilled water. Wipe dry and polish with a clean soft cloth. Place in a clean dry weighing

[1] Prepared by grinding 4 parts of hydrated lime with 10 parts of casein.

bottle and weigh. Fit the stem of a funnel of about 150 ml. capacity with rubber tubing and a pinch clip and fill with distilled water to within one quarter of an inch from the top. Immerse the slide again in chromic acid solution for a few moments, remove with forceps and wash with tap and distilled water and place in the distilled water in the funnel with a small part of the top of the slide protruding. Dry the forceps and hold the slide with its long edge parallel to the wall of the funnel without quite touching it. Run off the water by opening the clip to its fullest extent and transfer the slide to the weighing bottle. Weigh and so obtain the amount of water retained on the slide. Repeat the experiment, again starting by immersing the slide in chromic acid solution and average the results. Carry out duplicate determinations in a similar way with the soft soap and gelatine solutions, with the difference that after washing the slide with distilled water it is immersed in the soap or gelatine solutions in a beaker before placing in the funnel.

The results indicate that soap increases retention slightly and that gelatine has a much greater effect, i.e. gelatine is a better " sticker " than soap.

Protective Colloids and Stability of Suspensions. In the case of suspensions of lead arsenate or sulphur it is important to prevent the sedimentation of the solid particles, so as to ensure even distribution of a spray of known concentration. For this purpose materials which function as protective colloids or as dispersing agents are used and many substances of the spreader class are able to act in this way. Since the rate of fall of the particles is inversely proportional to the viscosity of the liquid medium, substances such as gelatine, which form dilute solutions of much greater viscosity than water, are able to delay sedimentation much better than soap, which gives solutions of viscosity very little greater than that of water. The spreader " sulphite lye ", on the other hand, probably acts by dispersing aggregates of amorphous particles and such materials are often included in proprietary pastes of lead arsenate, copper pastes and the so-called colloidal sulphurs.

Prepare a 0·8 per cent. suspension of lead arsenate paste (one which contains no added " spreader ") by vigorously shaking 4 gm.

of the paste with 500 ml. of water. Measure 100 ml. of the suspension into each of five cylinders and treat the contents as follows :

(1) Add 100 ml. of water.
(2) Add 100 ml. of a 1 per cent. solution of lime casein.
(3) Add 100 ml. of a 1 per cent. solution of soft soap.
(4) Add 100 ml. of a 0·8 per cent. solution of gelatine.
(5) Add 100 ml. of a 1 per cent. solution of sulphite lye.

Shake the cylinders again, allow to stand for several hours and observe the time taken for the lead arsenate particles to settle.

EMULSIONS AND EMULSIFYING AGENTS

An emulsion may be defined as a suspension of tiny particles of one liquid in another liquid in which it is insoluble. The suspended liquid is called the dispersed phase and the other liquid constitutes the continuous phase. An emulsion is thus only capable of dilution with the liquid of the continuous phase. Emulsions of the oil in water type (oil the dispersed and water the continuous phase) are of great importance in spraying, and the following experiments illustrate some of the physical properties of emulsions and aspects of importance relating to their preparation.

Breaking. Make a mixture of about equal volumes of petroleum oil and water in a test tube and shake vigorously until a milky liquid results. Note that on standing the oil globules quickly coalesce and that the water and oil separate into two layers.

This phenomenon is termed " breaking " the emulsion, and the liquids separate because the water molecules at the interface have a greater attraction for each other than for the petroleum molecules and vice-versa. It is important that the emulsions used in spraying should not break before application.

Action of the Emulsifier. To the mixture in the last exercise add a few ml. of a strong solution of soft soap (an emulsifier). Shake vigorously, allow to stand and observe that the emulsion is much more permanent. This is an oil in water type of emulsion.

The principal function of the emulsifier is to modify the properties of the interface between the dispersed and continuous

phases. The majority of spreaders which are capable of functioning as emulsifiers are compounds which lower the surface tension of water, and in so doing become more concentrated at the surface layer. This property is shown by compounds possessing a large molecule, in which one end of the molecule is water-attracting (the " polar " group) the other end being water-repelling (the " non-polar " group). In the last exercise adsorption of soap occurs at the interface, the polar group (–COOK) being held by the water, whereas the oil-soluble non-polar group (a long chain hydrocarbon group, e.g. $C_{17}H_{33}$) is held by the oil, thus producing a film which resists the tendency of the oil globules to coalesce.

Creaming. When an emulsion is allowed to stand "creaming" occurs, the cream being the true emulsion, which originally was suspended in the continuous phase. The creaming is due to differences in density between the dispersed and the continuous phases, the name having resulted from the analogy with the creaming of milk, where the fat rises in the natural emulsion of fat and serum. Tar oil-water emulsions, however, cream downwards since the oil has a greater density than that of water, while the usual petroleum oil emulsions cream upwards. In spraying practice, creaming must not be allowed to occur as it would result in the application of a spray of uneven concentration.

(1) Shake a mixture of equal volumes of olive oil and a solution of soft soap (about 0·5 per cent.) in a test tube and allow the emulsion to stand. Note the rise of the emulsified oil globules. Shake the tube and note that the emulsion may be reformed.

(2) Dilute a proprietary tar oil emulsion to 5 per cent. strength and allow to stand. Note that the tar oil globules gradually fall to the bottom of the vessel.

(3) Dilute a proprietary petroleum oil emulsion to $7\frac{1}{2}$ per cent. strength and pour the same volume (say 100 ml.) into each of three cylinders of obviously different diameters, (100, 250 and 500 ml. measuring cylinders will serve). Allow to stand and note that creaming is more rapid in the wider vessels since the globules conform to Stokes' law, i.e. the time taken is proportional to the length of the perpendicular path they have to traverse.

(4) Prepare a dilute petroleum oil emulsion by diluting about 8 ml. of a concentrated emulsion with water to 100 ml. and prepare a sample of similar dilution after adding 8 ml. of cresylic acid (a mixture of cresols consisting of an oily liquid of specific gravity 1·05). Pour the solutions into two cylinders of the same diameter and allow to stand. Note that the addition of cresylic acid—a liquid of greater density than water but completely miscible with petroleum, markedly retards the process of creaming and consequently stabilises the emulsion.

The above experiments illustrate that in spraying practice creaming must be prevented by the efficient agitation of the rather shallow vessels used as containers. The method of equalising the densities of the dispersed and continuous phases is also beneficial.

Inversion. Pour the emulsion obtained in the last exercise into a boiling tube, and add to it a weak solution of calcium chloride (about 0·1 per cent.) a few ml. at a time, shaking after each addition. Observe that an insoluble " buttery " scum of calcium soaps forms by interaction of the calcium chloride with the soap. When sufficient calcium chloride solution has been added, the scum rises to the surface leaving a clear watery layer below. The scum also contains the oil in an unemulsified condition, since the potassium soap films round the globules have been removed. Note that the emulsion will not reform on shaking. Pour off the scum with as little water as possible into a test tube, add a little more oil and shake vigorously. Note than an emulsion forms which differs from the original ; it is a water in oil emulsion.

Sodium and potassium soaps are soluble in water but not in oil, and always give emulsions of the oil in water type. On the other hand, calcium, magnesium and lead soaps are soluble in oil, but not in water, and give emulsions of the water in oil type. The latter type of emulsion, if used in spraying, would coat foliage with a film of oil, which would cause injury. Emulsifiers which form water-soluble calcium or magnesium salts are therefore unsuitable for the preparation of emulsions which have to be diluted with hard water, because of their tendency to favour inversion.

Simultaneous Preparation of Emulsifier and Emulsion ("Two-solution Wash "). Emulsification is greatly facilitated if the substance mainly responsible for emulsification can be formed in situ. For example, a fatty acid may be dissolved in petroleum and added to water containing dissolved caustic potash or soda. The soap which results, as it were within the petroleum, is more thoroughly dispersed through it than would result from simply mixing it with a solution of soap.

Dissolve 3 ml. of oleic acid in 10 ml. of petroleum oil, add this to 100 ml. of 1 per cent. sodium hydroxide contained in a bottle and shake. The emulsion which results can be added to 900 ml. of water, to form an emulsion containing approximately 1 per cent. of petroleum.

ADDENDA

Some chemical substances commercially obtainable, which find a use by the agriculturist or horticulturist and for which no convenient laboratory exercises have been worked out, include the following :

Derris Powder—is the ground-up root of a plant and is used as a contact poison against many insect pests of plants and also animal parasites, notably the warble fly. It contains the active principle rotenone, a white crystalline material almost insoluble in water, slightly soluble in mineral oils but soluble in most organic solvents.

Pyrethrum Powder—is prepared from the flower heads of certain species of pyrethrum. It contains the active principles known as Pyrethrin I and Pyrethrin II and is used as a contact poison against insect pests of plants.

Tar Oil Washes.—These oils which result from the distillation of tar are applied to fruit trees in the dormant state, i.e. as winter washes. They comprise creosote oils and anthracene oils of specific gravity greater than 1, consisting predominantly of hydrocarbons of the aromatic group, i.e. those associated with a structure with a benzene nucleus, and contain, in addition, constituents soluble in acid and in alkali. The former constituents are termed tar bases and the latter, tar acids or phenols.

Petroleum Oil Washes.—These mainly consist of saturated hydrocarbons, but unsaturated hydrocarbons may be present according to the source of the oil and the degree to which it has been fractionated, i.e. its boiling range.

The unsaturated hydrocarbons may, by reaction with sulphuric acid (sulphonation), be converted into acid soluble derivatives.

Selective Herbicides

A number of these have recently come into use on a field scale. These possess a high degree of potency and wind-borne dusts and sprays containing the active materials may damage neighbouring more sensitive crops, if applied during unsuitable weather conditions.

Dinitro-ortho-cresol (abbreviated to D.N.C. or D.N.O.C.).

This substance is a yellow dyestuff, usually marketed for spraying purposes as the sodium salt, with or without the addition of an " activator " such as ammonium sulphate, depending upon the crop and the nature of the weed. Usually it is sold as a concentrated paste as the dry material is inflammable.

This substance is also used as a winter (ovicidal) wash for fruit trees.

2 *Methyl—4 chloro-phenoxy-acetic acid* (abbreviated to M.C.P.A.).

2 : 4 *Dichloro-phenoxy-acetic acid* (abbreviated to D.C.P.A.).

Mixed with an inert base to allow of application as dusts, these substances are marketed under various names.

APPENDIX

REAGENTS

Acid mercuric nitrate. Dissolve mercury in twice its weight of concentrated nitric acid with application of heat, and dilute with an equal volume of water.

Alcohol-acetate solution (for use in turbidity method for determination of potassium in soil extracts). Dissolve 8 gm. of pure sodium acetate ($CH_3 . COONa . 3H_2O$) in 10 ml. of pure formalin and 10 ml. of glacial acetic acid. Add 220 ml. of ethyl alcohol (rectified spirits of wine). Store for a week before using.

N.B.—The formaldehyde in the formalin is sufficient to remove the amount of ammonia usually found in soil extracts.

Alkaline potassium iodide (for water analysis). Dissolve 70 gm. of potassium hydroxide and 15 gm. of potassium iodide per 100 ml. of solution.

Ammonia (8 per cent.). Dilute one volume of concentrated ammonia solution (sp. gr. 0·880) with three volumes of water.

Ammonia (2 per cent.). Dilute one volume of 8 per cent. ammonia solution with three volumes of water.

Ammonium chloride (for water analysis). The stock solution is made by dissolving 3·15 gm. of freshly sublimed pure ammonium chloride in ammonia-free water and making up to 1 litre at 15·5° C. The solution to be used in the test is made by measuring 10 ml. of the stock solution and diluting to 1 litre with ammonia-free water. (1 ml. = 0·00001 gm. of NH_3.)

Ammonium molybdate. Place 125 gm. of molybdic acid and 100 ml. of water in a litre flask, and dissolve the molybdic acid by the addition while shaking of 300 ml. of 8 per cent. ammonia solution. Add 400 gm. of ammonium nitrate and make up to the mark with distilled water. Shake well and add to 1 litre of nitric acid of specific gravity 1·19 (350 ml. of concentrated HNO_3 to 550 ml. water). Keep the solution at about 35° C. for twenty-four hours and then filter.

Ammonium molybdate-sulphuric acid solution (for colorimetric determination of phosphate). Dissolve 2·5 gm. of ammonium molybdate in 20 ml. of water at 60° C. Dilute 28 ml. of pure con-

centrated sulphuric acid to 80 ml. When the solutions are cool, mix by adding the molybdate slowly to the acid. Transfer to a 100 ml. flask and when cool make up to the mark. Store in an amber coloured bottle and keep for 3 days before using.

Ammoniacal zinc chloride. Dissolve 50 gm. of zinc chloride in about 500 ml. of water and add 125 ml. of concentrated ammonium hydroxide solution which will redissolve the precipitate first formed. Add 50 gm. of ammonium chloride and dilute to one litre.

Antimony trichloride (for vitamin A test). This is a saturated solution of antimony trichloride in dry chloroform. This reagent is usually purchased.

Basic lead acetate. Boil 264 gm. of litharge (PbO) with 464 gm. of neutral lead acetate in 1000 ml. of water for half an hour. Allow to cool, dilute to two litres, leave to settle and decant.

Benedict's solution. Dissolve the following with the aid of heat in enough distilled water to make about 800 ml. of solution :

Sodium citrate, 200 gm.
Sodium carbonate (cryst.), 200 gm.
or Sodium carbonate (anhydrous), 75 gm.
Potassium thiocyanate, 125 gm.

Filter and cool to room temperature.

Accurately weigh 18 gm. of pure (analar) crystalline copper sulphate, dissolve in about 100 ml. of distilled water and pour it slowly into the above solution with constant stirring. Add 5 ml. of a 5 per cent. solution of potassium ferricyanide and then distilled water to make the total volume 1000 ml.

Buffer solution for exchangeable hydrogen. To 25 ml. of 2 per cent. ammonia add about 800 ml. of distilled water and titrate with N./100 HCl, using methyl red as indicator. Calculate the volume required to make 1 litre of exactly N./100 and transfer this volume to a 1 litre graduated flask. Dissolve 53·5 gm. of ammonium chloride in distilled water and wash into the flask. Finally make up to the mark with distilled water.

Buffer substrate (for phosphatase test). This may be purchased in a convenient tablet form, or the solution may be prepared as follows. Dissolve 1·09 gm. of di-sodium phenyl phosphate and 11·54 gm. of " sodium veronal " in water, add a few drops of chloroform as an antiseptic and dilute to 1 litre. (The solution should be freshly prepared.)

Calcium bicarbonate (approximately 0·02N.). Prepare a saturated solution by passing carbon dioxide into a suspension of calcium

carbonate in a sparklet siphon. Filter the solution so obtained and dilute with one-third its volume of distilled water; the solution will be approximately 0·02N.

Congo-red silver nitrate papers (for detection of HCN). Immerse strips of filter paper for one minute in a 0·05 per cent. solution of Congo-red in water (made by diluting a 0·5 per cent. stock solution). Dry as quickly as possible, immerse in a 0·5 per cent. solution of silver nitrate and again dry quickly, excluding strong light as much as possible.

Diphenylamine reagent. Weigh 0·085 gm. of diphenylamine, mix with 50 ml. of distilled water and gradually add 450 ml. of concentrated sulphuric acid.

Fehling's solution. This is made up in two solutions.

(*a*) Accurately weigh 69·28 gm. of pure (analar) crystallised copper sulphate, dissolve in water and make up to 1 litre.

(*b*) Weigh 350 gm. of Rochelle salt (sodium potassium tartrate) and 100 gm. of sodium hydroxide, dissolve in water and make up to 1 litre.

For qualitative or quantitative tests equal volumes of these two solutions are mixed, the mixed solution being known as Fehling's solution.

Folin and Ciocalteu's phenol reagent (for phosphatase test). This may be purchased or prepared as follows. Dissolve 100 gm. of sodium tungstate and 25 gm. of sodium molybdate in 700 ml. of water, and treat with 50 ml. of syrupy (85 per cent.) phosphoric acid and 100 ml. of concentrated hydrochloric acid. Boil the mixture for ten hours under a reflux condenser in a 1500 ml. flask with a ground-glass connection. After the mixture has cooled, add 150 gm. of lithium sulphate and a few drops (usually 4 to 6) of bromine. Expel the excess of bromine by boiling the liquid for fifteen minutes, allow to cool (the reagent should now be golden yellow without any green tint), make up to 1 litre and filter.

Glyoxylic acid (reduced oxalic acid). Place 10 gm. of powdered magnesium in a flask and just cover with distilled water. Slowly add 250 ml. of saturated oxalic acid, cooling under the tap at intervals. Filter off the insoluble magnesium oxalate, acidify with acetic acid, dilute to one litre with distilled water and keep in a closed bottle containing a little chloroform.

Griess-Ilosvay reagent (for nitrite test). It consists of two solutions which are used in equal parts for the test.

(1) 0·8 gm. of sulphanilic acid in 100 ml. of 30 per cent. acetic

acid. (2) 0·5 gm. of α naphthylamine in 100 ml. 30 per cent. acetic acid. Dissolve with the aid of heat, filter and keep the α naphthylamine in a dark coloured bottle.

Indicators. (*a*) *Methyl Orange.* Dissolve 1 gm. in a litre of distilled water.

(*b*) *" Screened " methyl orange.* Dissolve 1 gm. of methyl orange and 2·5 gm. of indigo carmine in 1 litre of water. The colour change with this mixture is much sharper than with methyl orange, the change being from yellowish green (alkaline) to grey (neutral) and violet (acid).

(*c*) *Methyl red.* Dissolve 1 gm. in a small quantity of alcohol. Make up to a litre with alcohol, diluted with an equal volume of water.

(*d*) *Phenolphthalein.* Dissolve 5 gm. in 500 ml. of alcohol and dilute to a litre with distilled water.

(*e*) *Bromo-cresol green.* Dissolve 0·1 gm. by grinding in a mortar with 14·4 ml. of N./100 NaOH. Dilute with distilled water to 250 ml. The colour change is yellow (acid) to blue (alkaline). When used in titrations, titrate to a greenish blue colour (*p*H 4·8).

(*f*) *Bromo-thymol blue.* Dissolve 0·1 gm. by grinding in a mortar with 16 ml. of N./100 NaOH. Dilute with distilled water to 250 ml.

(*g*) *Chloro-phenol red.* Dissolve 0·1 gm. by grinding in a mortar with 23·6 ml. of N./100 NaOH. Dilute with distilled water to 250 ml.

(*h*) *Mixed indicator for soil testing.* Dissolve 0·1 gm. of bromo-thymol blue in 60 ml. of industrial methylated spirits and add 40 ml. of distilled water. Dissolve 0·05 gm. of methyl red in 45 ml. of methylated spirits by boiling and add 30 ml. of distilled water. Mix the two solutions and dilute with 525 ml. of distilled water. Adjust the *p*H to approximately 7·4 by the addition of N./10 NaOH.

(*i*) *Starch paste.* Add a little cold water to 1 gm. of soluble starch, make into a thin paste and wash into 50 ml. of boiling water. Boil for three minutes, add 10 gm. of sodium chloride, heat gently for one minute, cool and dilute to 100 ml.

Mayer's reagent. This may be purchased or prepared as follows. Dissolve 13·5 gm. of mercuric chloride in about 750 ml. of water in a litre flask, and slowly add 200 ml. of a solution containing 50 gm. of potassium iodide. Make up to the mark with distilled water.

Magnesia mixture. Dissolve 110 gm. of crystallised magnesium chloride and 140 gm. of ammonium chloride in 1300 ml. of water. Mix with 700 ml. of 8 per cent. ammonia solution, leave to stand for three days and filter.

Millon's reagent. See acid mercuric nitrate.

Nessler's reagent. This may be purchased or prepared as follows. Dissolve 62·5 gm. of potassium iodide in 250 ml. of distilled water, and gradually add with constant stirring, a cold saturated solution of mercuric chloride, until a very slight permanent precipitate remains. Dissolve 150 gm. of potassium hydroxide in 150 ml. of water, mix with the above and make the total volume up to 1 litre with distilled water. Keep in a closed vessel for about three days and then decant the clear liquid into a bottle.

Schiff's reagent. Prepare a weak aqueous solution of fuchin (magenta) and pass sulphur dioxide through the solution until just decolourised.

Schweitzer's reagent. Add sodium hydroxide solution to a solution of copper sulphate until a heavy light blue precipitate is formed. Filter off the cupric hydroxide and wash until free from alkali. Add concentrated ammonium hydroxide to the cupric hydroxide in just sufficient amount to dissolve it.

Sodium cobaltinitrite (qualitative test for potassium). This is best purchased as a 16 per cent. solution or it may be prepared as follows. Dissolve 50 gm. of cobalt nitrate and 300 gm. of sodium nitrite in water, acidify with 25 ml. of glacial acetic acid and dilute to a litre. Allow to stand for twenty-four hours, filter and keep in a dark coloured bottle.

Standard iron solution (for colorimetric determination of iron). Dissolve 0·352 gm. of pure ferrous ammonium sulphate ($FeSO_4 . (NH_4)_2SO_4 . 6H_2O$) in about 50 ml. of water and 5 ml. of concentrated sulphuric acid. Add 25 ml. of concentrated nitric acid to oxidise the ferrous iron to ferric. Heat gently to expel oxides of nitrogen, transfer to a 500 ml. flask and when cold dilute to the mark. This solution contains 0·0001 gm. of iron per ml. From this solution a more dilute standard is prepared containing 0·000001 gm. of iron per ml. by diluting 10 ml. to 1 litre with the addition of 15 ml. of concentrated sulphuric acid before making up to the mark.

Standard manganese sulphate solution (for colorimetric determination of manganese). Dissolve 0·144 gm. of pure dry potassium permanganate in about 100 ml. of water in a beaker. Add 10 ml. of concentrated sulphuric acid, heat the solution and reduce the permanganate by the careful addition of a solution of sodium sulphite. Boil to drive off the excess of sulphur dioxide, cool, transfer to a 500 ml. flask and make up to the mark. This solution contains 0·0001 gm. of manganese (Mn) per ml.

Standard phosphate solution (for colorimetric determination of phosphate in 0·3N. HCl soil extracts). Dissolve 0·2143 of pure anhydrous di-sodium hydrogen phosphate (Na_2HPO_4) in 0·3N. hydrochloric acid and make up the volume with the acid to 500 ml. This solution contains 21·4 parts of P_2O_5 per 100,000, which is equivalent (under the conditions of the test) to 50 parts of P_2O_5 per 100,000 of soil. Weaker solutions equivalent to 0·5, 5 or 10 parts P_2O_5 per 100,000 of soil are easily prepared from this stock solution.

Standard potassium solution (for turbidity determination of potash in 0·3N. HCl soil extracts). Dissolve 0·475 gm. of pure KCl in water and make up to 1 litre. This solution contains 30 parts of potash (K_2O) per 100,000, which is equivalent (under the conditions of the test) to 70 parts of K_2O per 100,000 of soil. From this stock solution, standards equivalent to 3 parts to 10 parts of K_2O per 100,000 of soil are easily prepared.

Stutzer's reagent. Dissolve 20 gm. of copper sulphate in 1 litre of water to which 2·5 gm. of glycerol are added. Add 10 per cent. sodium hydroxide solution until the mixture is just alkaline, filter off the blue precipitate of $Cu(OH)_2$ and wash with water containing glycerol (5 gm. of glycerol per litre) until the washings are free from alkali. Rub up the precipitate in a mortar with water containing 10 per cent. of glycerol, to form a gelatinous paste of such consistency as can be measured in a pipette.

Sulphuric acid (5 per cent. for fibre determination). Measure 27 ml. of the pure concentrated acid (sp. gr. 1·84) and pour into about 800 ml. of water. Cool and dilute to a litre.

Uffelmann's reagent. To a 1 per cent. solution of phenol add very dilute ferric chloride solution until the solution becomes coloured an amethyst-violet.

Wijs iodine solution. This may be purchased or prepared as follows. Dissolve 8·5 gm. of iodine and 7·8 gm. of iodine trichloride in separate portions of about 450 ml. of glacial acetic acid. Mix the solutions and make up to a litre.

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